EVALUVATION OF IMMUNOMODULATORY ACTIVITY OF AQUEOUS EXTRACT OF *CASSIA OCCIDENTALIS* LEAVES *IN WISTAR RATS*

A Dissertation submitted to
THE TAMIL NADU Dr. M.G.R. MEDICAL UNIVERSITY,
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In partial fulfillment of the requirements for the award of the Degree of
MASTER OF PHARMACY
IN
BRANCH –VI - PHARMACOLOGY

Submitted by
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MAY – 2017

CERTIFICATES

CERTIFICATE

This is to certify that the dissertation entitled**“ Evaluvation of Immunomodulatory Activity of Aqueous Extract of *Cassia Occidentalis* By Leaves in Wistar Rats”** being submitted to The TamilNadu Dr. M.G.R Medical University, Chennai was carried by **Mr. Habeeb Rahman kp** to The Tamil Nadu Dr. M.G.R Medical University, Chennai in partial fulfillment for the degree of **Master of Pharmacy** in **Pharmacology** is a bonafied work carried out by candidate under my guidance and supervision in the Department of Pharmacology, Karpagam College of Pharmacy Coimbatore – 32.

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DECLARATION

I hereby declare that this dissertation **" Evaluvation of Immunomodulatory Activity of Aqueous Extract of *Cassia Occidentalis* By Leaves In Wistar Rats"** submitted by me , in partial fulfillment of requirements for the degree of **Master of Pharmacy** in **Pharmacology** to The Tamil Nadu Dr.M.G.R Medical University, Chennai is the result of my original and independent research work carried out under the guidance of **Dr. C. Senthil Kumar., M.Pharm.,PhD** Associated Professor , Department of Pharmacology ,Karpagam College of Pharmacy , Coimbatore -32

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CHAPTER I

1. INTRODUCTION

1.1 Immune system

Immune system is a complex organization of white blood cells, antibodies, and blood factors that protects the body from foreign invaders, while simultaneously maintaining self-tolerance (Baniyash, 2006). A series of specialized epithelial and stromal cells also provide the anatomic environment which regulate various functions of immune system by secreting several critical factors. The immune system is a network of cells, tissues, and organs that work together to defend the body against attacks by “foreign” invaders. These are primarily microbes (germs)—tiny, infection-causing organisms such as bacteria, viruses, parasites, and fungi. Because the human body provides an ideal environment for many microbes, they try to break in. It is the immune system’s job to keep them out or, failing that, to seek out and destroy them.

The immune system is a system of biological structures and processes within an organism that protects against disease. Disorders of the immune system can result in autoimmune diseases, inflammatory diseases, cancer and immunodeficiency[1]. Immunomodulation is a procedure which can alter the immune system of an organism by interfering with its functions; if it results in an enhancement of immune reaction, it is named as an immunostimulative drug which primarily implies stimulation of non-specific system. Immunosuppressant implies mainly to reduce resistance against infections, stress and may occur on account of environmental or chemotherapeutic factors. Immunostimulation and immunosuppressions both need to be considered in order to regulate the normal immunological functioning. Hence both immunostimulating agents and immunosuppressing agents have their own standing, so search for better agents exerting these activities is becoming the field of major interest all over the world.

A number of Indian medicinal plants and various ‘Rasayana’ have been claimed to possess Immunomodulatory activity[2].The use of plant products as immunomodulators is still in a developing stage. A variety of plant-derived materials such as polysaccharides, lectins, peptides flavonoids and tannins have been

reported to modulate the immune system[3]. Since ancient times, several diseases have been treated by administration of plant extracts based on traditional medicine[4]. Natural adjuvants, synthetic agents, antibody reagents are used as immunosuppressive and immunostimulative agents. But there are major limitation to the general use of these agents such as increased risk of infection and generalized effect throughout the immune system[5].

The immune system that humans have evolved is, however, not perfect and we discuss some of these imperfections at the end of this chapter. The immune system is a very effective killing machine, and if it goes wrong it can cause severe disease and even death of its host. To cover these latter areas we first consider how the immune system is able to discriminate between what needs to be eliminated and what does not – particularly in the case of adaptive immunity, which has evolved to recognize molecular structures largely at random. We then introduce the different ways in which the immune system can cause damage if it becomes directed not to infectious agents, but to otherwise harmless targets, including many inert substances around us and within the tissues of the host itself. We discuss the problems of transplants (some of which can even attack their hosts) and why the immune system fails to reject malignant tumors (cancer). Finally, we turn from problems to solutions and introduce two areas in which either the intact immune system or components of immunity can be harnessed for our own benefit and from which tools can be derived to treat disease.

Different types of cells and molecules are involved in the initiation of innate and adaptive immune responses although, as mentioned above, their interaction is essential in defense against most infectious agents. So what do the innate and adaptive arms of immunity do in general terms? Broadly speaking we can view some components of the innate immune system as being involved in the detection of “harmful” things that represent “danger” to the organism, such as general classes of microbes that may have infected the host. Other components then endeavor to eliminate the microbe. In contrast, the adaptive immune system can discriminate very precisely between individual microbes, even of the same type, but can generally only make a response if it has been informed by the innate system that what is being recognized is “dangerous”. If so, adaptive responses may then help to eliminate the microbe, if it has not already been eradicated during the earlier innate response.

Recognition of infectious agents is essential for any form of immunity and thus for host defense against them.

The key components of the innate immune system include cells such as phagocytes and soluble molecules such as complement. This work together to sense the presence of infection. The recognition of potentially dangerous microbes usually leads to the generation of inflammation, familiar to us all. One way of viewing this is that the innate immune systems of multi-cellular organisms can generate .alarm. signals in response to danger, and that some of these signals cause inflammation

The key components of the adaptive immune system are the lymphocytes. It is convenient at this stage to divide these into two main groups (other types do exist). One group is the T lymphocytes (T cells) which have evolved to interact with other cells. The other is the B lymphocytes (B cells) which are the precursors of cells that can make soluble antibodies. The recognition of molecules from infectious agents by lymphocytes is mediated by their specialized antigen receptors, which are not present on cells of innate immunity

The lymphocyte is the basic cell responsible for both humoral and cellular immunity. This cell in resting stage is small (6μm in diameter), with a high nuclear-to-cytoplasmic ratio, indicative of its lack of activity. A pool of recirculating lymphocytes passes from the blood into the lymph nodes, spleen, and other tissues and back to the blood by the major lymphatic channels such as the bone marrow and the thymus.

The bone marrow hemopoietic stem cells are the ultimate origin of erythrocytes and all leukocytes, including the lymphocytes. Many lymphocytes pass through the thymus where they become processed by the hormonal microenvironment prior to release. These lymphocytes are now called as thymus-derived lymphocytes, T lymphocytes, or T-cells the majority of the bone marrow-derived lymphocytes which do not enter or become processed by the thymus are called B cells.

1.2 Immune mechanisms

The introduction of foreign substance (antigen) into the body provokes an immune reaction & for this it is essential that the body recognize it as “non self”.

Most antigens are first ingested & concentrated by the macrophages, & later passed to the nearly lymphocytes. The immune response is initiated by the

interaction of the antigen with the receptors on the surface of the lymphocytes, and the response may be of true types

1.2.1 Humoral immunity

Humoral immunity is the aspect of immunity that is mediated by secreted antibodies, produced in the cells of the B lymphocyte lineage (B cell). B cells express a unique B cell receptor (BCR), in this case, an immobilized antibody molecule. The BCR recognizes and binds to only one particular antigen.

A critical difference between B cells and T cells is how each cell "sees" an antigen. T cells recognize their cognate antigen in a processed form - as a peptide in the context of an MHC molecule, while B cells recognize antigens in their native form. Once a B cell encounters its cognate (or specific) antigen (and receives additional signals from a helper T cell (predominately Th2 type), it further differentiates into an effector cell, known as a plasma cell.

1.2.2 Cell mediated immunity

Cell-mediated immunity is an immune response that does not involve antibodies but rather involves the activation of macrophages, natural killer cells (NK), antigen-specific cytotoxic T-lymphocytes, and the release of various cytokines in response to an antigen. Historically, the immune system was separated into two branches: humoral immunity, for which the protective function of immunization could be found in the humor (cell-free bodily fluid or serum) and cellular immunity, for which the protective function of immunization was associated with cells.

1.2.3 Innate Immune Responses

Immune cells, non-immune cells and non-cellular systems all participate in initiating an innate immune response. Why is it called “innate”? It’s innate because it depends on intrinsic systems that are built into your body to recognize danger and there is no learning or adaptation involved[8].

1.3 Receptors of the Innate Immune Response

In order to detect PAMPs or DAMPs, cells need tools to recognize them. These tools are protein receptors that can be found on the cell surface as well as internally. In general, they are called pattern recognition receptors or PRRs. These receptors come in families consisting of multiple members. Receptors that recognize PAMPs include the Toll-like receptors (TLRs), the C-type lectin receptors (CLRs), the NOD-like receptors (NLRs), RIG-I-like receptors (RLRs) and invariant T cell receptors.[9, 10]

DAMP receptors are not so clear-cut. TLRs have been implicated as well as the receptor for advanced glycation endproducts (RAGE). Also the purinergic receptors that recognize ATP would also fall into this category.[11]

1.3.1 Toll-like Receptors

These receptors are found on most cells of the body. They recognize a variety patterns associated with a number of pathogens including virus-associated nucleic acids; bacterial-associated cell wall components, protein, ribosomal RNA and DNA; and protozoan-associated proteins. The majority is found extracellular, but a number are also found intracellular. When stimulated they activate the transcription factor NFκB, which is essential for activating a cell's immune functions and set off a signal cascade via MAP kinase (a phosphorylating enzyme).[12]

1.3.2 C-type Lectin Receptors

These receptors are specialized in recognizing carbohydrate structures, such as the sugar mannose, which is a common component of fungal cell walls[13].Thus, these receptors are found on the cell surface. Though much of the literature involves their expression on immune cells, reports of CLR variants on non-immune cells can also be found[14]. On the phagocytic cells, it is known that they can participate in endocytosis, the engulfment of particles or pathogens and respiratory burst[15]. Some also appear to initiate signal cascades similar to TLRs leading to NFκB and MAP kinase activation, but it also appears that they can work in concert with TLRs, enhancing or inhibiting their function[16].

1.3.3 NOD-Like Receptors

These receptors are found in the cytoplasm of cells. Traces of their expression is found in most organs of the body and it is probably safe to say that most immune cells express at least some members of the NLR family. These receptors are designed to detect intracellular bacteria and, possibly, endogenous stress molecules and allow the cell to produce one of the most potent inflammatory mediators, Interleukin (IL)-1β[17].

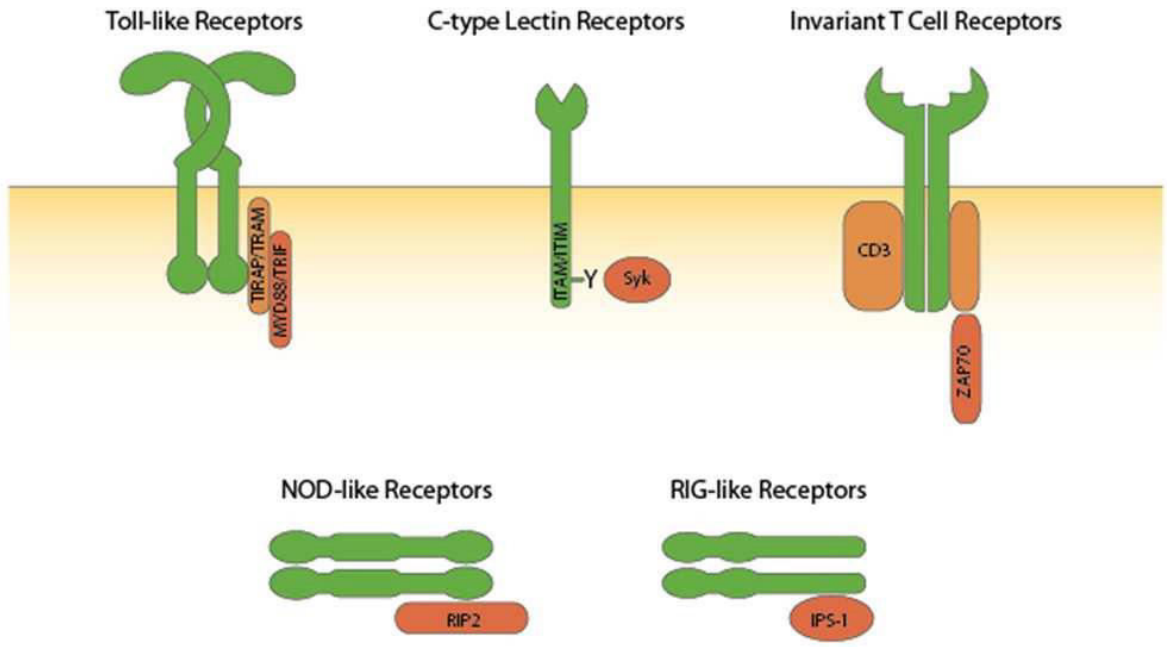
1.3.4 RIG-I-like Receptors

Like NLRs, RLRs are also found in the cytoplasm of a cell. Instead of detecting bacterial products, these receptors help detect viral infection.[18] They do this by binding to RNA produced during viral replication. Working together with nucleic-acid detecting TLRs, they lead to NFκB, MAP kinase activation and activation of

Interferon regulatory factor (IRF) transcription factors[19].The IRF transcription factors are necessary to produce cytokines specialized for the control of viral infections. Cytokines are small, secreted proteins used as messengers between cells, which alert surrounding immune cells about danger.

Fig No. 1.3A: Invariant Receptors of the Innate Immune System

1.4 Immune Cells of the Innate Response



Invariant Receptors of the Innate Immune System

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Under epithelial layers are resident macrophages, neutrophils, dendritic cells, NK cells, mast cells and a number of T cell-related cells[20].

1.4.1 Macrophages

The name macrophage is derived from Greek, meaning “large eaters”. Their main function is to phagocytize (engulf) pathogens and particles. It does this by

wrapping its plasma membrane around particles until they are enveloped and pinched off to form an endosome inside the cell. Once inside the cell, the endosome merges with a lysosome that contains en Macrophages also have the ability to generate a “respiratory burst”, which is a release of oxygen radicals that damage surrounding pathogens and cells. They also can alert and attract other immune cells through inflammatory cytokine release[21].

1.4.2 Neutrophils

Neutrophils are the main foot soldiers of the innate immune response and are certainly the most abundant. They also have a wide arsenal of tools to deal with invaders. Like macrophages, neutrophils can phagocytize particles, release a respiratory burst and produce inflammatory cytokines. Unlike macrophages, neutrophils have the internal caches of anti-microbial substances called granules[22].

1.4.3 Dendritic Cells

Dendritic cells are also phagocytic cells, but they have the special ability of initiating an adaptive immune response (will be discussed later).[23] Unlike neutrophils and macrophages, Dendritic cells or DCs are not simple foot soldiers. Instead, they function more as spies and provide intelligence about invaders to T cells through a phenomenon called “antigen presentation” and through cytokine production[24].

1.4.4 NK Cells

The NK stands for Natural Killer and the name implies their function. These cells, however, do not kill pathogens directly. Instead, these cells have the ability to recognize when other cells are harboring internal pathogens using special receptors and then kill them. Situations where this might occur is during viral and mycro bacterial infections. These pathogens easily reside in host cells, finding ways to block lysosome fusion and their own destruction[25].

1.4.5 Mast Cells

Mast cells are the cells that are responsible for the classic signs of inflammation, which include redness, swelling and heat. Though well known for their association with allergy, they also can detect PAMPs and DAMPs through receptors and become immunologically active. Mast cells exert their functions mainly through cytokine and granule release. Unlike neutrophils, which release antimicrobial substances, mast cells release histamine and heparin. Histamine is well known for

its vasodilator function and ability to allow fluid to leak between cells, causing redness and swelling. It also causes inflammatory itching by triggering neurons (unmyelinated C-fibers) responsible for the itch feeling. Heparin prevents blood coagulation[26.27].

1.4.6 T Cell-like cells

Most T cells are part of the adaptive immune response as they have adaptive T cell receptors (receptors that learn to recognize pathogens). NK T cells and γδ T cells, however, use invariant T cell receptors (receptors that do not rearrange) or semi-invariant T cell receptors and participate in the innate immune response.

NK T cells are similar to the NK cells mentioned above. Not so much in function, but more in how they look. These cells share many of the same surface protein markers. NK T cells, however, do not kill compromised cells. Instead, they are quick cytokine producers. In doing so, they quickly notify all surrounding cells that there is problem when they recognize PAMPs presented to them via dendritic cells[28].

The γδ T cells are important for innate immune reactions and the adaptive immune response as they have invariant and variant T cell receptors. Their precise function remains unclear, but they can secrete cytokines and, like the NK T cells above, participate in alerting and strengthening local immune responses[29].

i) Non-cellular Systems of the Innate Immune Response

Besides cells, there are also defenses in your body that are ready to react to pathogens as soon as they are encountered, much like booby traps. These systems rely on small proteins that are found within the bodily fluids.

ii) Complement System

The liver synthesizes the proteins of the complement system and they work in concert to aid in phagocytosis, bacteria lysing and immune cell attraction. One can visualize it as a self-assembling machine that starts to assemble as soon as the first proteins are bound and in place. The complement “machine” is known to be initiated by three different pathways: the classical pathway, the alternative pathway and the lectin pathway. The classical pathway is triggered when antibodies are bound to a pathogen. The alternative pathway is triggered when the victim is unable to block the cascade (normal cells can, while pathogens cannot). The lectin pathway uses free

lectin proteins (lectins are proteins that bind sugars) to bind sugars associated with bacterial cell walls).

iii) Acute Phase Proteins

These proteins are also produced by the liver and especially during inflammation when proinflammatory cytokines are produced. Many are designed to coat pathogens and have chemotactic properties (have the ability to attract cells). Some inhibit microbial growth by sequestering iron from the environment. The lectins from the lectin pathway of complement activation are considered acute phase proteins[30].

iv) Anti-microbial Peptides

Often called “defenses”, these peptides function as natural antibiotics and our produced by cells that guard the external surfaces and internal surfaces such as the skin and the gastrointestinal system. In the skin, the main sources are keratinocytes, mast cells, neutrophils, sebocytes and eccine epithelial cells. In the intestines, one of the main producers are the Paneth cells of intestinal crypts[31].

v) Adaptive Immune Responses

The adaptive immune response is what gives individuals long-term immunity to a pathogen after vaccination. Instead of relying on germ-line encoded receptors for the recognition of pathogens like the innate immune system, it depends on the development of receptors that can recognize any unique molecular characteristic of pathogens[32]. The molecules that can be recognized are called antigens. The classical definition of an antigen is any molecule that can provoke the development of antibodies. A better, and less-confusing, definition is a molecule that can be recognized by the adaptive immune system. The molecules are often protein peptides (small pieces of protein). But, they can also be sugars, lipids and other small molecules under the right circumstances. The main players of the adaptive immune response are the T cells (both T helper cells and cytotoxic T cells) and the B cells.

vi) The Bridge Between Innate and the Adaptive Responses: Dendritic Cells

During the innate immune response, the first steps are taken to initiate an adaptive immune response. The main cells responsible for this step are the DCs that we described earlier. As we mentioned before, DCs are a phagocytic cell type. This means that they have the ability to engulf pathogens/particles in endosomes and later fuse these vesicles to lysosomes for destruction. The process, however, does

not stop here. Instead of just disposing of the pathogen/particle waste, the DC, instead, uses these parts to educate T helper cells about the pathogens. It does this by traveling from the location where it picked up its parcel to the local lymph node, where it finds T helper cells. Once there, it presents the pathogen-associated peptides on its surface using molecules called MHC class II molecules and provides information to T cells about how it should respond using surface molecules called co stimulatory molecules and cytokines. Educating T helper cells is the first step towards initiating an adaptive immune response.

1.5 T helper Cells and Their Education

T helper cells or the cells are crucial cells in the adaptive immune response and they are characterized by a surface protein called, CD4. They hold the key to initiating the functions of cytotoxic T cells[33] and B cells[34]. Furthermore, they can also increase the efficacy of macrophages.

The cells interact with the MHC class II/peptide complexes presented by antigen presenting cells through its receptor, called the T cell receptor (TCR). If a T cell has never before seen antigen, it is called a naïve T cell. In this situation, the T cell will need instruction from a professional antigen 13 presenting cells, usually a DC, about how to perform its function. DCs do this through cell surface proteins call 0co-stimulatory molecules and through cytokine expression. This process is consists of three main signals. The first signal is the antigen recognition; the second signal is co-simulation and the third cytokine exposure. This whole process is referred to as “priming” of the naïve T cell. Once primed, the T cells begin to divide; a process that is referred to as expansion or proliferation[35].

The most important set of co-stimulatory molecules is CD80 or CD86 on the DC and CD28 on the T cells. This second signal is necessary to tell the cell that there is a problem. If signal one is given without this second signal, the T cell will assume that the antigen is actually harmless and become non-responsive in a process called “anergy”[36]. Only a DC that has encountered a PAMP or another danger signal will express CD80 or CD86 on its surface reassuring the Th cell that there is, indeed, a problem.

Signal three is the secretion of cytokines of the DC. There are several cytokines important for the cell education. They most important ones are IL-4, IL-12, IL-6, TGFβ and IL-10. Th cells will differentiate into different types of the cells depending on which cytokines prevail. The main types of the cells are T helper 1

(Th1) cells, T helper 2 (Th2) cells, T helper 17 (Th17) cells, and induced regulatory T cells (iTreg).

1.5.1 The Cell Subtypes

Each the cell subtype has its own unique set of skills. One could almost see differentiation as an occupation. Just like an athlete will choose to develop her body and a scientist will choose to develop her mind. In humans, these choices are reflected at the level of gene transcription and protein expression. The athlete will stimulate muscle growth and the scientist develops the cerebral cortex of the brain. It's the same for the cell differentiation. The four main subtypes of the cells are listed. There are, however, rare forms that have been observed that are not listed and The cells, much like humans, can fall into gray areas between the stereotypes.

i) T helper 1 Cells

The Th1 path is chosen when T cells are exposed to IL-12 during priming. Th1 cells are characterized by the production of the cytokine, interferon-γ (IFNγ) and the expression of the master transcription factor, T-bet. The1 cells are experts at gearing the immune response towards to the control of internal pathogens like viruses and mycobacteria, which reside internally in macrophages. They perform this function by initiating cytotoxic T cell responses, helping macrophages to become more effective, by helping B cells to produce certain types of antibodies. These functions are executed, in part, 14 through IFNγ exposure; however, some require cell-cell contact and will be explained in more detail later[37].

ii) T helper 2 Cells

Th2 cells are created during exposure to high amounts of IL-4. This leads to the expression of the Th2-associated master transcription factor, GATA3. Th2 cells are also characterized by the production of IL-4 (indeed, the same cytokine needed to create them). These cells are designed to skew the immune system towards a humoral immune response (antibody response) that can deal with parasite infection. Unfortunately, Th2 responses are also the ones associated with allergy development as well. Th2 cells do their work by effectively helping B cells and encouraging specific forms of antibodies. This is done through a combination of IL-4 exposure and cell-cell interactions.

iii) T helper 17 Cells

The Th17 subtype is the most recently described of the Th subtypes. It is most effective at controlling extracellular bacterial and fungi responses, like those found during intestinal food poisoning or during a yeast infection. Its creation is dictated by the cytokines IL-6 and TGFβ and this leads to the expression of the master transcription factor, RORγt. Th17 cells produce the cytokine IL-17. IL-17 production is one of the main facilitators of their function and it encourages surrounding cells to increase neutrophil migration. Neutrophils are excellent phagocytic cells with many bacterial killing tools.[38]

iv) Induced Regulatory the cells

To those just learning about the immune system, the existence of the following the subtype may be confusing. I Treg are designed to counter the functions of other immune cells. Why? The reason is that immune responses are highly damaging to surrounding tissues and, without them, immune responses would spiral out of control.

That said; these cells are induced by DCs when they are exposed to high amounts of IL-10 or TGFβ. This causes the expression of the master transcription factor, Foxp3. In turn, iTreg produce IL-10 or TGFβ. IL-10 and TGFβ are what is called “anti-inflammatory” cytokines. They have the ability to limit the functions of immune cells. IL-10, for instance, lowers Th1 and Th17 responses and reduces macrophage efficacy. TGFβ encourages apoptosis (induced death of cells), prevents cell division and lowers phagocytosis.[39]

1.6 Cytotoxic T cell Responses

Th cells are not the only kind of T cell. Cytotoxic T cells (CTLs), characterized by the surface marker CD8, are not to be missed and are essential for the elimination of viral infections. The function 15 of a CTL is found in its name. “Cyto” refers to cell and “toxic” means just how it sounds. These cells are “cell toxic” and kill other cells. In many ways, they are similar to the NK cells and NK T cells of the innate immune system. However, they do not use invariant receptors to recognize problems in other cells, but instead use an adaptive system.

CTLs, like Th cells, have a TCR. This means that they can detect unique peptides presented to them by other cells. In the case of Th cells, these are MHC class II molecules presented via DCs. In the case of CTLs, they are MHC class I

molecules. During an infection, as we earlier mentioned, DCs will travel to the lymph node and present samples of the intruder to the T cells. This is also happens for CTLs. However, despite the presence of all the priming signals, priming will be suboptimal. CTLs need an additional signal, jokingly called “the license to kill”. This signal is given by a Th1 cell through the production of a cytokine called IL-2, which stimulates CTL expansion; and through an interaction between the Th1 cell and the DC via CD40 on the DC and CD 40 legend on the Th1 cell, which makes
the DC more effective at priming CTLs33. Once a CTL is primed and active, it has the ability to kill.

As you can see, CTL activity is highly controlled to ensure that they react only to pathogen associated peptides. The reason is that MHC class I can be expressed by every cell type in the body. MHC class I on a cell is like a sign advertising the health of the cell. The cell is constantly displaying samples of the proteins it’s making. If an active CTL recognizes one of these samples as being of viral origin, it kills that cell; eliminating a viral host.

1.7 Adaptive Humoral Immune Responses

The word “humor” means fluid in Latin and, therefore, humoral immune responses relate to noncellular
Systems found in the bodily fluids. We’ve already discussed non-cellular components of the innate immunity; however, in immunology most people are not referring to these non-cellular systems when they use the term “humoral immune response”. Instead, they are referring to the immune response mediated by antibodies and this is part of the adaptive immune response.

The cell behind antibody responses is the B cell. Naïve B cells of the immune system produce rudimentary antibodies (see below) until other cells activate them. B cells, unlike the T cells, are not required to interact with DCs; instead B cells reside in lymphoid tissues and fish for antigens that they recognize using their B cell receptors or BCR. The BCR looks like a surface bound antibody and once it binds a molecule, the B cell engulfs it and much like the phagocytes, digests it. Just like the DC, the B cell will then present pieces of the antigen to Th cells using MHC class II molecules. Primed and activated Th cells, which recognize the presented peptides, are then able to “help” the B cell through a 16 CD40-CD40 ligand interaction. The Th cell also provides cytokine signals to tell the B cell which kinds of antibodies it should make.

This process is reminiscent of the priming process of Th cells. Signal one is the MHC class II/peptide and TCR interaction between the B cell and the T cell. Signal two is the co stimulatory help provided by the T cell in the form of CD40-CD40 ligand interactions. And, signal three is the cytokine message provided by the T cell.

Helped B cells will then further differentiates into plasma cells, which can produce massive quantities of antibodies.

1.8 Antibodies

Antibodies by themselves, cause very little harm. However, their strength lies in their ability to tag a molecule as harmful and block molecular functions. Antibodies enhance the functions of the innate immune system. They can bind to pathogens and particles to initiate the complement system and induce phagocytosis. They can also block/neutralize molecular interactions. Examples of this function would be an antibody that blocks the toxic effects of diphtheria toxin or antibodies the block viral binding sites to cells. Antibodies also interact directly with cells and can change their function by binding to specific antibody receptors found on the surfaces of immune cells[40].

An Antibody is a small protein structure produced by B cells. It is also called an immunoglobulin (Ig). It looks like a “Y” and it is formed from four separate proteins. Each tip of the “Y” recognizes and sticks to the antigen, meaning that each antibody can bind two similar antigens. A single arm is called a Fab (Fragment, antigen binding) fragment. The base of the “Y” is called the Fc (Fragment constant) region and, while the Fab fragments dictate the specificity of the antigen binding, the Fc region dictates the type of antibody or isotype. The antibody isotype is dictated by the prevalent cytokines in the environment as well as additional danger signals that the B cell experienced while being helped by the Th cell.[41]

1.8.1 Rudimentary Antibodies: IgM and IgD

The first types of antibodies that a B cell can produce are IgM and IgD. The “M” and “D” refers to different classes of the Fc region. IgM is found as a pentamer, with five individual IgM antibodies bound by their Fc regions in the center forming a star. They are effective at complement activation. IgD is found as a monomer and its function is undefined. However, it has the ability to bind mast cells via an Fcδ receptor (δ for D) and induce anti-microbial peptide secretion.

1.8.2 IgG

IgG antibodies are found as monomers and they are very potent at stimulating immune responses. They are capable of neutralization, inducing phagocytosis in macrophages and neutrophils via Fcγ receptors (γ for G), activation of complement, and also the activation of NK cells (also via Fcγ receptors).

1.8.3 IgE

IgE antibodies are monomers. They are known to cause mast cell degranulation via binding of Fcε receptors (ε for E). They are induced during parasite infection and, unfortunately, also during allergy.

1.8.4 IgA

IgA is found as a dimer of two antibodies attached via their Fc regions. It is involved with mucosal defense: found in gastrointestinal system, the respiratory systems. They are particularly effective at neutralization of microbes and toxins.

How the Adaptive Response Strengthens the Innate Response

Once the adaptive immune system has formed a response, the body has a long-term record of the invading pathogen in the form of long-lived plasma cells, memory T cells (not covered here) and antibodies. This is why vaccination is so important. It allows your body to create an adaptive immune response against an invader without having to truly become infected.[42]

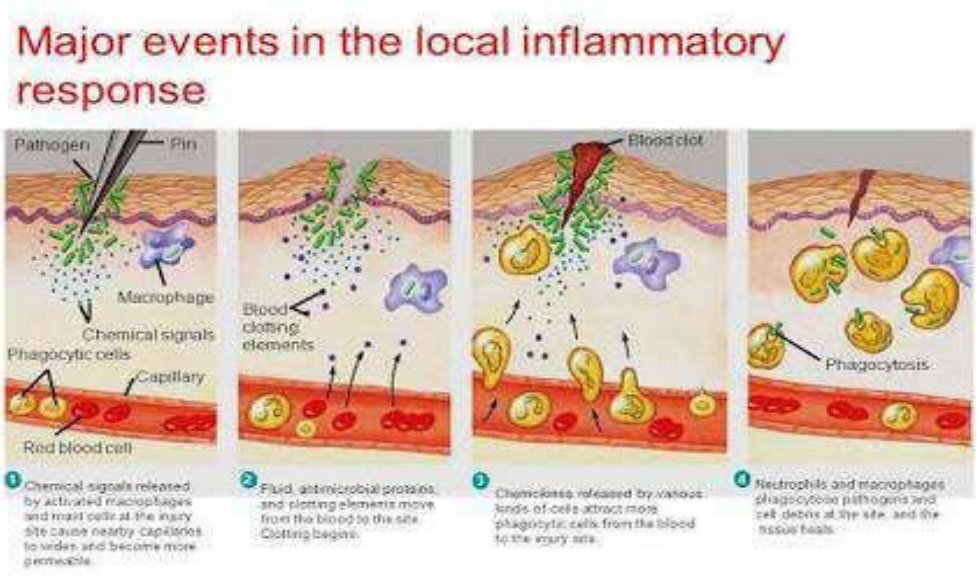
When a body encounters a pathogen for the second time, it's a completely different situation than the first encounter. During a second infection, T cells drawn to the inflammation site will have knowledge to help macrophages, recruit more neutrophils, and kill infected cells. Antibodies will benow present to assist complement activation, the phagocytosis of particles, and even kill microbes. The response will be quicker and more effective.

Though separating the two types of responses: innate and adaptive, helps with learning; it can also become an obstacle to seeing the immune response as a complex, dynamic system. It is important when looking at an immunological problem to consider the host's previous history as it has so much influence on the immune response.

1.9 Inflammatory response[43]

In vivo systems, which involve the whole animals, provide the most natural experimental condition, due to their complexity; in vivo systems have a myriad of unknown and uncontrollable cellular interactions that add ambiguity to the interpretation of data. At the other extreme are in vitro systems, which defined populations of lymphocytes are studied under controlled and consequently repeatable conditions.

Fig. No 1.3B: Major Events in the local inflammatory response.



1.10 Immune mechanisms

It is medicated by thymus-derived cells known as T cells, which interact with the antigen to reduce lymphocytes. The immune response is an essential defense mechanism against the invasion of the body by bacteria. Sometimes the immune reaction to an antigen does not only produce antibodies which damage the antigen, but may also damage body tissue causing hypersensitivity reactions. These reactions are of four types.

i) Type [Ig E or regain. dependant] reaction

In this the antigen antibody combination occurs on the surface of the most cells & polymorphonuclear leucocytes, & releases pharmacologically active substances. This reaction is mediated by the IgE antibodies, which sensitize the cells.

The onset of type I reaction is within 1-2 minutes, maximal at 15-30 minutes, lasts 1½ to 2 hours.

ii) Type II [cytotoxic tissue – specific antibody] reaction in this the antibody, in the presence of complement, reacts with on antigenic component of all cell or tissue, & results in cell lysis or damage. This reaction is mediated by IgM or Ig G antibodies.

iii) Type III [Immune – complex disease, Arthus reaction]

This is caused by circulating antigen-antibody complexes formed in conditions of slight antigen excess, together with complement which can cause tissue destruction directly & also by attracting polymorphonuclear neutrophills to the site.

iv) Type IV [cell-mediated delayed hypersensitivity reactions]

This immune reaction is mediated by sensitized circulating lymphocytes of the T-cell type reacting with antigen. The immune globulins of the type I, II & III reactions are not involved in most of the manifestations of the reaction are produced by the lymphokines which are soluble factors produced by the sensitized lymphocytes on contact with antigen phagocytosis.

This type of reaction takes 1-2 days to develop after antigen exposure, and reaches it is peak at 48-72 hours. E.g. Organ – transplant rejection, contact dermatitis, tuberculosis lung reaction and tuberculin skin reactions.

v) Inflammatory Response

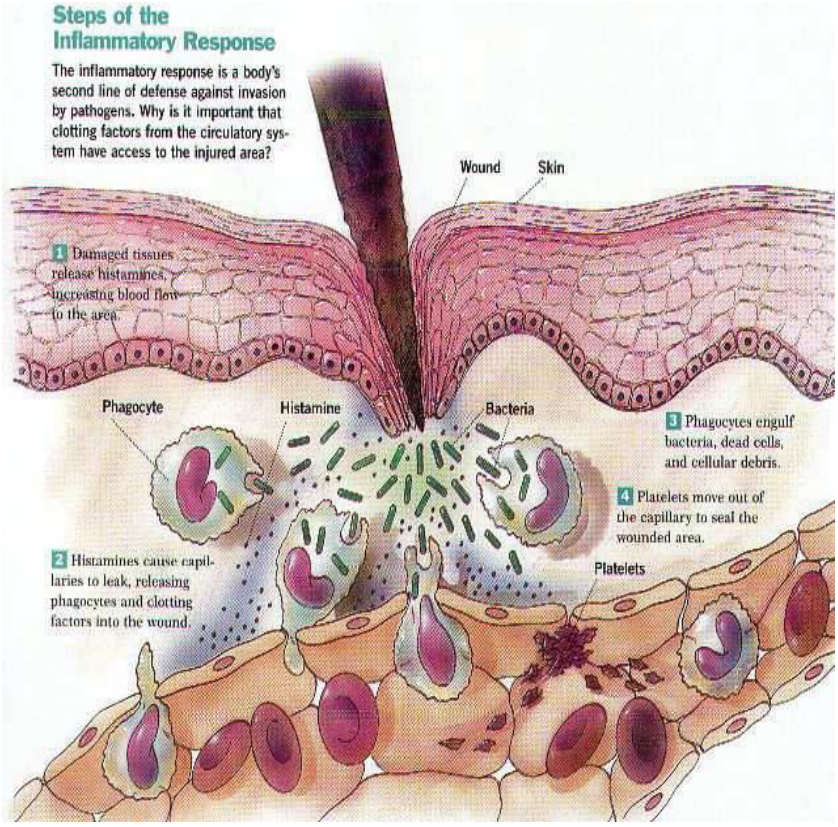
The final consideration in discussion of defense mechanisms is how the nonspecific factors discussed above combine in what is termed the inflammatory response to combat an invasion by pathogens.

If the microbe is able to activate and fix complement by the alternate pathway, the chemotactic complement-derived factors released to attract leukocytes to the site, and anaphylatoxin also causes the de granulation of Tissue leukocytes to the site and an aphylatoxin also because the de granulation of tissue 20 basophils called must cells. These in turn release histamine and serotonin, which cause constriction of smooth muscles (e.g., in bronchioles and blood vessels) and increased capillary permeability, which promoted the passage of plasma and leukocytes into the affected tissue. The leukocytes pass through the junction between the capacity endothelial cells in response to the passage of plasma and leukocytes into the affected tissue. The leukocytes pass through the junction between the capillary endothelial cells in response to the chemotactic influence of the complement cleavage fragments. Migration continues until the phagocytes

encounter complement fixed to the microbial surface, which causes adherence and facilitates engulfment. If antibodies specific for the microbe are present, they opsonize it and alsoincrease complement fixation. This greatly enhances leukocyte adherence and promote Phagocytosis. The plasma also contains other micro bicidal substances, which may inhibit replication and growth of pathogens and modulate the subsequent immune response. The symptoms of the inflammatory response are local swelling, erythema (reddening), and local and systemic heat. The local swelling is due in part to the accumulation of large numbers of phagocytic cells at the site of infection. The increase in temperature and the erythema are due to the increased blood flow to be local site, enzymatic activity, and the release of bacterial endotoxin

Steps In Inflammatory Response

Fig. No 1.3C: Steps in inflammatory response



1.11 Experimental System:

In vivo systems, which involve the whole animals, provide the most natural experimental condition, due to their complexity; in vivo systems have a unknown and uncontrollable cellular interactions that add ambiguity to the interpretation of data. In vitro systems can be studied effectively, yet they have their own limitation, the most notable of which is their artificiality, for example, providing antigen to purified B cells in vitro does not stimulate maximal antibody production unless T cells are present.

1.12 Experimental Animal Model.

A study of the immune systems in vertebrates requires suitable animal models. The choice of an animal depends on its suitability for attaining a particular research goal. If large amounts of antiserum are sought, a rabbit, goat, sheep, or horse might be an appropriate experimental animal. If the goal is development of a protective vaccine, the animals chose meet be susceptible to the infectious agent so that the efficacy of the vaccine can be assessed. Mice or rabbits can be used for vaccine development if they are susceptible to the pathogen.

For most basic research in immunology, mice and Rats have been the experimental animals of choice. They are easy to handle, are genetically well characterized and have a rapid breeding cycle. The immune system of Rats and mice has been characterized more extensively than that of any other species.

CHAPTER II

2. REVIEW OF LITERATURE

Natural immunomodulators *Cassia occidentalis*

Mohammed Musa Suleiman et al (2014)[47] reported anthelmintic activity of *cassia occidentalis*. Guieri senegalensis standard technique was used to detect secondary metabolism to prove in vitro anthelmintic activity, using Hatch inhibition test (HETT) and larval development inhibition assay.

Olufunke Bolatito et al.(2014)[48] reported antimalarial screening of Spondian mombio, senna occidentalis and Musa sapientum against vibrio cholerao1, the tannins flavanoids alkaloids responsible for the anti malarial activity by minimum inhibitory concentration and minimum bactericidal and fungicidal concentration. Vibrio cholerae O1 can cause large epidemic of cholera with high mortality. This study investigated the phytochemical and antimicrobial properties of extracts of leaves of Spondias mombin and Senna occidentalis and stem sap of Musa sapientum against two epidemic strains of V. cholerae O1 (BA O1 and CVC O1). Aqueous and ethanolic extracts of Spondias mombin and Senna occidentalis were obtained using soxhlet extraction while the stem sap of M. sapientum was obtained fresh. The filtrates were dried at 40 C and stored at 4oC. The crude extracts were subjected to phytochemical analysis using standard methods. In vitro antimicrobial studies were investigated using microbroth dilution method to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). Phytochemical analysis revealed the presence of tannins, saponins, alkaloids, anthraquinones, flavonoids, cardiac glycosides, phenols and phlobatannins. Aqueous and ethanolic leaf extract of Spondias mombin and aqueous leaf extract of S. occidentalis have vibriocidal activities with antimicrobial activity showing that S. mombin water extract (SMWE) had MIC (41.56mg/ml); and MBC (83.13mg/ml) against both BA O1 and CVC O1 respectively while the ethanolic extract (SMEE) had MIC (83.13mg/ml); and MBC (166.25mg/ml). Senna Occidentalis water extract (SOWE) had MIC (166.25mg/ml); and MBC (332.50mg/ml) against BA O1 and CVC O1 respectively. Both aqueous and ethanolic extracts of M. sapientum and ethanolic extract of S. occidentalis were not effective in vitro on the epidemic strains tested. 31 Spondias mombin and Senna

occidentalis could be useful in drug research and development because of the photochemical that they possess.

Komal et al.(2013)[49] reported that allellopathic influences of *cassia occidentalis* on growth of zea mays. For evaluating the allelopathic effect of leaf and flower extracts of common wasteland weed *Cassia occidentalis* L. on seed germination and seedling growth of Triticum aestivum L. Both extracts inhibited the growth of wheat seedlings under laboratory conditions with more pronounced effect in case of flower extract. A dose-dependent decrease in growth and dry weight of seedlings was observed, however, at lower concentrations, enhancement in seedling growth was also recorded.

Tanimu et al.(2012)[50] Acute toxicity test was conducted in a report with *Cassia occidentalis* and found that this plant did not show any hazardous symptoms or death . With the sub acute treatment, the *Cassia occidentalis* does not change body weight gain, consumption of food and water and the profiles of hematological and biochemical. Also, no changes were seen in macroscopical and microscopical aspect of organs in the animals. Thus they conclude that acute or sub acute administration of *Cassia occidentalis* is not toxic.

Sadiq et al.(2012)[51] to evaluated and reported phytochemistry and anti microbial activity of *cassia occidentalis* , preparation of anti-bacterial medium and activity tested by using arE well diffusion method. Leaves of *Cassia occidentalis* were extracted with ethanol and water. The extracts were used to carry out antimicrobial screening in vitro on staphylococcus aureus, pseudomonas aeruginosa, Escherichia coli, salmonella typhi, shigella spp. Chromatographic separation was carried out on the active extracts, and the efficacy of the resulting fractions was tested against the susceptible organism. Some of the extracts indicated significant inhibitory activity against the tested organisms. General phytochemical screening was done on the ethanol, water extracts and fractions. Ethanol extract revealed the presence of Tannins, Saponins, Cardiac glycoside, Terpenoids and Anthraquinones while the fraction revealed the presence of Tannins, Terpenoid and Anthraquinones. This result might explain the ethnobotanical use of the plant for the treatment of dysentery, gastro internal disorder, constipation and Typhoid fever.

Mohammed et al.(2012)[52] reported the anti microbial activity in leaves of *cassia occidentalis* against staphylococcus aurous cultured in nutrient agar,

incubation and determined minimum inhibitory concentration and minimum bactericidal and fungicidal concentration.

Srinath Reddy et al. (2012)[53] reported anti-anxiety and antidepressant activity of ethanolic and aqueous extracts of *Cassia occidentalis* leaves in rodents. Exposing the rats to unfamiliar aversion in different methods like elevated plus maze model and actophotometer anti-anxiety activity was tested. Less aversion fear elicits anti-anxiety activity. Antidepressant activity was analyzed by despair swim test and tail suspension test. Reduced immobility time elicits antidepressant activity. They conclude that ethanolic and aqueous extracts of *Cassia occidentalis* leaves possess anti-anxiety and antidepressant activity. Anxiety and Depression are widespread psychiatric disorders affecting around 5% of the population. Furthermore, it is difficult to predict which patient will respond to any given treatment. In the traditional systems of medicine, many plants and formulations have been used to treat anxiety and depression for thousands of years. The present study was designed to evaluate the anti-anxiety and antidepressant activity of the ethanolic and aqueous extracts of *Cassia occidentalis* leaves in rodents. Antianxiety activity was tested by exposing rats to unfamiliar aversion in different methods like elevated plus maze model and actophotometer. The results infer that reduced aversion fear elicits anti-anxiety activity. The antidepressant activity was tested by using despair swim test and tail suspension test. The results infer that reduced immobility time elicits antidepressant activity. It was concluded that ethanolic and aqueous extracts of Cassia occidentalis leaves having anti-anxiety and antidepressant activity. Ethanolic extract of Cassia occidentalis leaves showing more significant activity over the aqueous extract.

Ravikumar et al. (2011)[54] reported antioxidant activity of ethanolic extract of *cassia occidentalis* leaves against carbon tetra chloride induced oxidative stress in Wistar rats. The efficacy of ethanolic extract from *Cassia occidentalis* against CCl4 induced oxidative stress was tested using wistar albino rats. The antioxidant activity was assessed by monitoring the levels of lipid peroxides, antioxidant enzymes like glutathione peroxidase, glutathione reductase, glutathione- Stransferase, superoxide dismutase and catalase, and non-enzymic antioxidants like reduced glutathione, vitamin-C, vitamin-E, cereloplasmin and uric acid in the liver tissues. Administration of CCl4 increased the level of lipid peroxides decreasing the activities of enzymic and nonenzymic antioxidants. Pre-treatment with ethanolic extract significantly prevented the alterations induced by CCl4 and maintained a near normal antioxidant

status. Decreased activities of enzymes in CCl4 intoxicated rats and their reversal in the ethanolic extract treated rats shows the potency of ethanolic extract in combating CCl4 induced oxidative stress.

Abirami Dhandapani et al (2011)[55] reported larvicidal and pupicidal potential of *Cassia Occidentalis* was analyzed in a study against the larvae of Anopheles Stephensi. The ethanol extract of *Cassia Occidentalis* were found to be more effective against larva and pupa respectively. The smoke toxicity study was also conducted and identified that it was more effective against the Anopheles stephensi. Smoke exposed gravid females ovipositor fewer eggs when compared to those that were not exposed. A simple High Performance Thin Layer Chromatographic (HPTLC) method has been developed for the analysis of flavonoid in ethanol extracts of *Cassia Occidentalis*. The amount of flavonoid in the extracts has been estimated by comparing the peak area using the standard. The proposed HPTLC method was found to be simple, faster and reliable for analysis of flavonoid. Cassia Occidentalis were the dominant invasive weed in the campus of Bharathiar University India. Their allelopathic activity has greatly affected the phytodiversity in the campus. With the view of their huge biomass prospecting, the larvicidal potential of ethanol extract of Cassia Occidentalis was tested against the larvae of Anopheles Stephensi. The ethanol extract of Cassia Occidentalis were found most effective with LC50 value of 60.69%, 64.76%, 67.78%, 70.56%, 92.21% of I, II, III, IV and pupa respectively. The smoke toxicity was more effective against the Anopheles stephensi. Smoke exposed gravid females oviposited fewer eggs when compared to those that were not exposed.

Karpakavalli et al[56]. reported analgesic and antipyretic activity of *cassia occidentalis*. Ethanol and water extracts of Cassia occidentalis leaves were screened in mice which were induced b acetic acid and tested for hot plate and tail immersion assay. The ethanol and water extracts possess antinociceptive and antipyretic properties. Highest inhibition dose was found to be as 300 mg/kg56.

Prabh et al. (2011)[57] reported anti diabetic activity of leaves of *cassia occidentalis* using alloxan induced hyperglycemia and quantitatively estimated blood glucose level. Many plants synthesize substances that are useful to the maintenance of health in humans and other animals. These include aromatic substances, most of which are phenols or their oxygen-substituted derivatives such as tannins. Many are secondary metabolites, of which at least 12,000 have been isolated — a number

estimated to be less than 10% of the total. In many cases, substances such as alkaloids serve as plant defense mechanisms against predation. Diabetes mellitus often simply referred to as diabetes—is a condition in which a person has a high blood sugar (glucose) level as a result of the body either not producing enough insulin, or because body cells do not properly respond to 34 the insulin that is produced. Insulin is a hormone produced in the pancreas which enables body cells to absorb glucose, to turn into energy. Cassia occidentalis is an annual shrub. The leaves, roots & entire plant as such has been used in various countries like China, Brazil, Sri Lanka & India in the treatment of the variety of ailments such as malaria, liver diseases, fungal infections etc. Thorough literature survey for the chemical nature of the plant reveals that the plant contains quinines, flavonoids, saponins and alkaloids. The ethno medical information reveals the plant has been used to treat diabetes.

Emmanuel et al. (2010)[58] reported that anti diabetic activity of leaves of *cassia occidentalis* in streptozotocin induced diabetics in rats, a dose dependent study calculated. Acute toxicity study and experimental induction of diabetics in rats. *Cassia occidentalis* Linn is extensively used in the indigenous and folklore medicine systems to treat several illnesses. However adequate characterization of hypoglycemic activity of *C.occidentalis* has not yet been done. The scientific evaluation of its hypoglycemic activity was, therefore, explored and also compared with the effect of a standard hypoglycemic drug, Glibenclamide. In the present study methanol fraction of *C.occidentalis* leaves (COLMF) was tested against streptozotocin-induced diabetic rats. Adult male albino Wistar rats, weighing 150-200g, were randomized into control and experimental groups. Experiment group rats were induced diabetes by a single intraperitoneal injection of streptozotocin (STZ). Treatment with COLMF at different doses and times following in normal and diabetic rats significantly reduced the blood glucose level to normal in diabetic rats (99.68±3.57). Hemoglobin, glycosylated hemoglobin, hepatic glycogen, lipid peroxidation, antioxidants enzymes (TBARS, HP, SOD, CAT, GPx VitC, VitE, and GSH) and hepatic marker enzymes (ALT, AST, ALP, ACP) were also evaluated in normal and diabetic rats. Oral administration of COLMF significantly and dose-dependently normalized the above mentioned parameters near to normal in STZ-diabetic rats ($p<0.05$). Histopathological examination showed that COLMF extract protected the pancreatic tissue from STZ-induced damage.

Bilal Bin-Hafeez et al.(2001)[59] reported that Immunosuppression ,cyclophosphamide (CP) was administered intraperitoneally in a single dose of 50 mg/kg b.w. Body weight, relative organ weight, lymphoid organ cellularity, hemagglutination titer (HT); plaque forming cell (PFC) assay and quantitative hemolysis of SRBC (QHS) were analyzed in animals. It has suppressive effectson lymphoid organ weight and cellularity and other parameters of humoral immunity.

CHAPTER III

3. AIM AND OBJECTIVE

The effective drugs are not available for the treatment of certain infections like AIDS, hepatitis, and other viral infections. For other infections the drug (mainly antibiotics) being used are becoming ineffective due to development of microbial resistance, necessitating the search for newer drugs. Any such new drug will be available only at an exorbitant cost due to the product patent norms under WHO agreement

In Siddha, Ayurveda and other ancient systems of medicine, many plants and plant preparations are reported to be useful in the treatment of infections. When screened by modern scientific methods these preparations did not show any immunomodulatory activity.

These drugs may not probably act directly upon the microbes. Instead may stimulate the body's defense mechanism (immune system) and thereby help to cure the infection.

Hence by screening herbal drugs and their extracts for their immunostimulant property it may be possible to get effective, cheaper new molecular entity for the treatment of various infections.

It may be hoped that such type of drugs will not produce microbial resistance, since they do not act on the microbes and will not have adverse side effects since they are from (natural) plant origin.

This work is to prove the Immunomodulatory property of *Cassia occidentalis* in Wistar rats by studying the Delayed Type Hypersensitivity (DTH), Humoral Antibody titre (HA), Total Leukocyte Count (TLC), and Differential Leukocyte Count (DLC) In the Aqueous Extract of *Cassia Occidentalis* leaves extract.

CHAPTER IV

4. PLAN OF WORK

Literature review

↓

Selection of plant

↓

Collection of plant

↓

Authentication of plant

↓

Powdered the leafs

↓

Extraction of powered plant leafs

↓

Phytochemical screening

↓

Acute toxicity studies

↓

Evaluation of immunomodulatory activity

↓

Statistical Analysis

↓

Result and Discussion

↓

Conclusion

CHAPTER V

5. PLANT PROFILE

5.1 Description of the Plant

Botanical name	:	*Cassia occidentalis*

5.1.1 Taxonomic Hierarchy[8]

Kingdom	:	*Plantae*
Subkingdom	:	*Angiosperm*
Super division	:	*Eudicots*
Class	:	*Dicotyledonae*
Subclass	:	*Rosidae*
Order	:	*Fabales*
Family	:	*Fabacae*
Genus	:	*cassia*
Species	:	*Cassia occidentalis*

5.1.2 Vernacular names

Common name	:	*Coffee senna*
Tamil	:	*Nattam takarai*
Hindi	:	*Kasunda*
Kannada	:	*Kolthogache*
Sanskrit	:	*Kasamarda*
Malayalam	:	*oolanthakara*

5.2 Botanical Description [44]

C.occidentalis is an erect, somewhat branched, smooth, semi-woody, fetid herb or shrub, 0.8 1.5 m tall, taproot, hard, stout, with a few lateral roots on mid-section. This plant species varies from a semi-woody annual herb in warm temperate areas to a woody annual shrub or sometimes a short-lived perennial shrub in frost free areas. The stem of the plant is reddish purple. The young ones are 4-sided, becoming rounded with age. Leaves are alternate, even pinnately compound, each

one with 4–6 pairs of nearly sessile, opposite leaflets, with a fetid smell when crushed, each leaflet 4–6 cm long, 1.5–2.5 cm wide, ovate or oblong, lanceolate with a pointed tip and fine white hairs on the margin. The rachis has a large, ovoid, shining, dark purple gland at the base. Stipules are 5–10 mm long, often leaving an oblique scar. Inflorescence is a compound of axillary and terminal racemes. The flower is perfect, 2 cm long with 5yellowish green sepals with distinct red veins and 5 yellow petals. The fruit is a dry, dehiscent, transversely partitioned, faintly recurred, laterally compressed, sickle shaped legume (pod), 7–12 cm long, 8–10 mm wide, with rounded tip and containing 25–50 seeds. Seeds are oval shaped, 3.5–4.5 mm wide, flattened; pale to dark brown, slightly shiny, smooth and with a round pointed tip.

Fig. No **5A:** ***Cassia occidentalis***

Fruit Seeds

5.3 Chemical constituents[45]

Achrosin, aloe-emodin, emodin , anthraquinones), anthrones, apigenin, aurantiobtusin, campesterol, cassiollin, chryso-obtusin, chrysophanic acid, chrysarobin, chrysophanol , chrysoeriol, emodin, essential oils, funiculosin, galactopyranosyl, helminthosporine , islandicine, kaempferol, lignoceric acid, linoleic acid, linolenic acid, mannitol, mannopyranosyl, matteucinol, obtusifolin, obtusin, oleic acid, physcion , quercetin, rhamnosides, rhein, rubrofusarin, sitosterols, tannins, and xanthorine

5.4 Parts used

Leaves, roots and seeds.

5.5 Properties and Used[46]

- Decoction of plant is used as hysteria, in dysentery and other stomach troubles.
- Leaves is mixed with calcium hydroxide is applied to dresses.
- Leaves powder is mixed with salt and onion poultices to gunea worms.
- Ointment of leaves is used as skin diseases.
- Decoction of leaves is used as blood purifier.
- Leaves internally and externally used skin diseases..

CHAPTER VI

6. MATERIALS AND METHODS

6.1 Chemicals and Drugs:

- The Sheep Red Blood Cells (SRBCs) were procured from U WIN Life science,Malappuram
- The Levamisole (Cipla Limited- India) was purchased from local pharmacy, Malappuram.
- The Humoral antibody test, TLC, Differential leukocytes tests were performed in UWIN Life Sciences. All chemicals were procured from UWIN Life sciences,Malappuram.

6.2 Collection of Plant Materials:

The dried leaves of Cassia occidentalis belonging to the family Fabaceae were collected from the Botany Central Council for Research in Ayurvedic and Siddha Govt. of India (NO:UWAU/351/16) and authenticated by Chelladurai.V research officier Botany Central Council for Research in Ayurvedha and Siddha Govt. of India

6.3 Preparation of Aqueous Extract[60]

The leaves will be cleaned with deionizer water, oven dried at 50^{0C} for 48 hours and powdered in a grinder. The plant material (200 gm) will be sequentially extracted with water (2000 ml) by using Soxhlet apparatus for 24 hours at a temperature not exceeding the boiling point of the solvent. The obtained extracts will be filtered by using Whatmann No. 1 filter paper and then concentrated under vacuum at 40^{0C} by using a rotary evaporator. The extract will be then lyophilized (Allied Frost lyophilized) to powdered form at 55^{0C} under vacuum conditions. The extractive value of the extracts (percentage yield) will be calculated. The residual extracts will be stored in refrigerator at 4^{0C} in small and sterile plastic bottles.

6.4 Acute Toxicity Study[61]

Acute toxicity for aqueous extract of leaves will be done according to the Organization of Economic Co-Operation and Development (OECD) guideline No: 423.The overnight fasted rat is weighed and selected. The extracts will be dosed in a stepwise procedure, with the use of 6 animals of a single sex (normally females) per step. Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step (i.e. no further three additional animals at the next higher or lower dose level). The starting dose level is selected from one of four fixed levels (5, 50, 300, and 2000) and it should be that which is most likely to produce mortality in some of the dosed animals. . Hence 200 mg/kg and 400 mg/kg b.w of *cassia occidentalis* extract were taken as effective doses for evaluation of immunomodulatory activity.

6.5 Expeimental Animals

Wistar rats (average body weight 150-200g), used from in house laboratory. The animals were maintained under standardized environmental conditions (22-28^{o}C , 60-70% relative humidity, 12 hr dark/light cycle) in animal house, Department of uwin life science malappuram. The animals were provided with standard mouse chow (SaiDurga Feeds and Foods, Bangalore, India) and water *ad libitum*. All animal experiments were conducted during the present study got prior permission from Institutional Animal Ethics Committee (IAEC approved) and following the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) constituted by the Animal Welfare Division, Government of India (No:-KU/IAEC/M.pharm/171)

6.6 Experimental Protocol:

24 rats were divided into four groups of six animals each.

Group-I: Control:

Group-II: Cassia occidentalis aqueous extract was administered at a dose 200mg/kg/day by oral route for 14 days

Group-III: Cassia occidentalis aqueous extract was administered at a dose of 400mg/kg/day by oral route for 14 days

Group-IV: Standard – Levamisole was administered at a dose of 50mg/kg/day by oral route for 14 days

6.7 Experimental Setup

The animal model is required to study the following

1. Delayed type hypersensitivity (DTH) response
2. Humoral antibody (HA) titer
3. Total leukocyte count
4. Differential leukocyte count

1. Determination Of Delayed Type Hypersensitivity Response (DTH)[23]

The fresh sheep blood was collected from U WIN life science. It was washed three times with normal saline via centrifugation. The suspension was adjusted to 1 $X10^8$.The animals were immunized by injecting 0.1 ml of SRBCs suspension, containing 1X 10^8 cells (1.0 X ^{108SRBC}/ml) intraperitonially, on day 0. On Day 8, after immunization the thickness of the right hind footpad was measured using a Vernier caliper. The rats were then challenged by injection of 1 X 10 8sub SRBCs in the left hind footpad. The footpad thickness was measured again after 24 hours of challenge. The difference between the pre- and post-challenge footpad thickness, expressed in mm was taken as a measure of the DTH response. The following formula to be used to measure the DTH response.

$$\frac{\text{(Left foot pad challenged with antigen-Right foot pad control)}}{\text{Left foot pad challenged with antigen}} \text{ X100}$$

2. Humoral Antibody Titer[62]

The animals were immunized by injecting 0.1 ml of SRBCs suspension, containing 1X 10^8 cells, intraperitoneally, on day 0. Blood samples were collected in micro centrifuge tubes from individual animals of all the groups by retro orbital vein puncture on day 10. The blood samples were centrifuged and the serum separated. Antibody levels were determined by the hemagglutination technique.

2.1 Method for Serial dilution

This was performed by using 96 wells (12x8) U bottomed titre plate. The wells were marked from I to XII. In the first (I) and last well (XII) 25 micro liter of serum collected from treated animals was added and inactivated at 56 degree Celsius for 30 minutes. Afterwards to all the wells except well number XII, 25 micro

liter of PBS was added.25 micro liter was taken from first well and added to 2nd well again 25 micro liter from second well was taken and added to third well and continued the same procedure up to well number XI. After this 25 micro liter of sample from well number XI was discarded. Finally 25 micro liter of 1% SRBC was added to all the wells and was kept at room temperature for two hours.

Observation:

The button formation was observed. The well which is previous to the well showing button formation is considered as Antibody titer.

Table No:1 Dilution

Well no	Dilution(antibody titer)
1	2
2	4
3	8
4	16
5	32
6	64
7	128
8	256
9	512
10	1024

3.Total Leukocyte Count[63]

3.1 W.B.C diluting pipette:

It has got three graduations. Two graduations 0.5 and 1 are present on the stem of the pipette and the third mark 11 is placed just above the bulb. Blood is drawn up to mark 0.5 and the rest of the bulb is filled by sucking up diluting solution up to the mark 11, the bulb of the pipette is so constructed that it holds exactly 20 times the volume of fluid contained in the stem of the pipette up to mark 1.Although fluid is drawn up to 11, the dilution of the blood will be 20 because the last part of the fluid remains locked up in the stem and is not available for dilution.

3.2 Counting chamber:

The ruling area consists of 9 square millimeters. The central of the smallest squares are separated by triple lines in which RBC will be counted. The side of each square for counting WBC is ¼ mm.

3.3 Diluting fluid for WBC (Turks fluid)

Commonly the fluid is made up as follows:

Glacial acetic acid -1.5ml

1%solution of gentian violet in water -1ml

Distilled water -98ml

The glacial acetic acid hemolysis the red cells, while the gentian violet stains the nucleus of Leukocytes

3.4 Method of counting W.B.C

The white cells are counted in four corners of 1 square millimeter ruled area on both sides. The white cells are recognized by the retractile appearance and by the slight color given to them by the stain contained in the diluting fluid. The cells touching the left side and upper side of boundary line are not counted.

3.5 Calculations:

The area of the smallest square	=	1/16 mm^3square
Volume of smallest square	=	1/160 mm^3
Total number of square counted	=	16×4=64
Total number of cells counted	=	X
64/160 mm^3 of diluted blood contains	=	X cells
So, 1 mm^3 of diluted blood contains	=	160/64 × X cells
1 mm3 of undiluted blood contains	=	160/64 × 20 × X cells

4 Differential Leukocyte Counts[64]

A thin blood film was made on a clean, dry, glass slide. It was dried fixed and stained to differentiate the different types of leukocytes. Hundred leukocytes were counted and percentage of different leukocytes was calculated

4.1 Composition of leishman's stain

It contains a mixture of methylene blue and eosin dissolved in acetone free methanol.

4.2 Procedure

A thin blood film was made on a clean dried glass slide. It was dried and stained with leishman's stain solution. The drop of leishman's stain was counted & 2 minutes was allowed to fix the blood film. Fixation means nucleus and various cellular organs will be fixed without any damage to the cells or cellular organs. After 2 minutes double the quantity of distilled water was added over the slide and waited for 7 minutes. In the meantime the stain will initiate the chemical reaction. The acidic dye eosin will initiate various acidophilus structures and some neutrophyllic granules and basic dye will stain structure like nucleus, basophilic granules, and cytoplasm of the lymphocyte and monocytes. After 7 minutes the slide was washed in a slow stream of water later it was dried in air. One drop of cedar wood oil was placed over the film. The cells were identified and entered into 100 squares. This gives the % of different types of leukocytes present in rat blood.

CHAPTER VII

7. RESULTS AND DISCUSSION

Effects of Test Extracts and Standard Drug on DTH Response in rats Using Sheep's RBCs as Antigen. The effect of test extract and standard drugs on the DTH response in wistar rats using SRBCs as antigen, administration of aqueous extract of Cassia occidentalis at the dose of 200mg/Kg and 400mg/Kg and Levamisole 50mg/Kg treatments which were given orally for 14 days showed significant increase in paw edema compared to control group. The standard drug Levamisole showed the maximum increase in paw edema volume compared to all groups. The results are shown in below

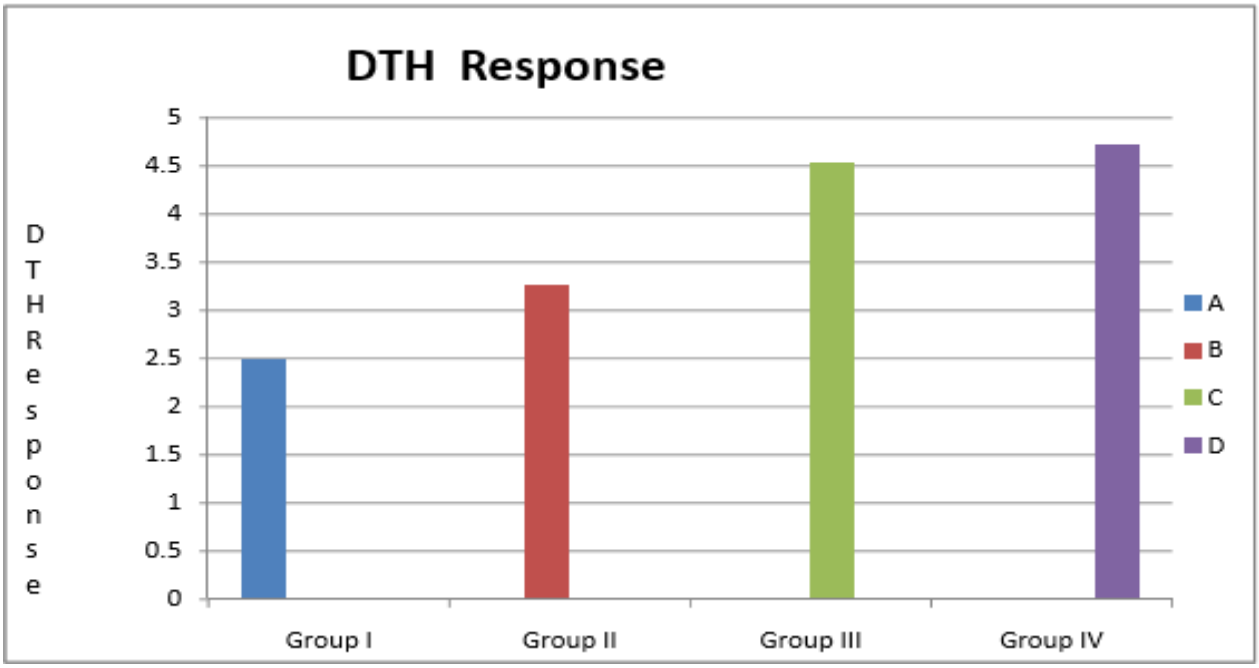
Table no.7.1: Effects of Test Extracts and Standard Drug on DTH Response in Rats Using Sheep's RBCs as Antigen.

Group	Treatment	Dose	DTH Response (mm) mean paw edema
I	Control		2.48±0.235
II	Test extract I	Cassia occidentalis 200 mg/kg	3.25±0.2658*
II	Test extract II	Cassia occidentalis 400 mg/kg	4.53±0.2416**
III	Standard	Levamisole 50mg/kg	4.71±0.1560***

Dunnett test and p values as significant* if $p<0.05$, highly significant** if $p<0.01$ and extremely highly significant*** if $p<0.001$ as compared to control

7.1A Delayed Type Hypersensitivity Reaction

Fig. No.7.1A: Delayed type hypersensitivity reaction



Group I : Control

Group II : Cassia occidentalis 200 mg/kg

Group III: Cassia occidentalis 400 mg/kg

Group IV: Levamisole 50 mg/kg

Fig. No.7.1B **Paw edema observed in animals after injecting sheep's RBC Control Animal**

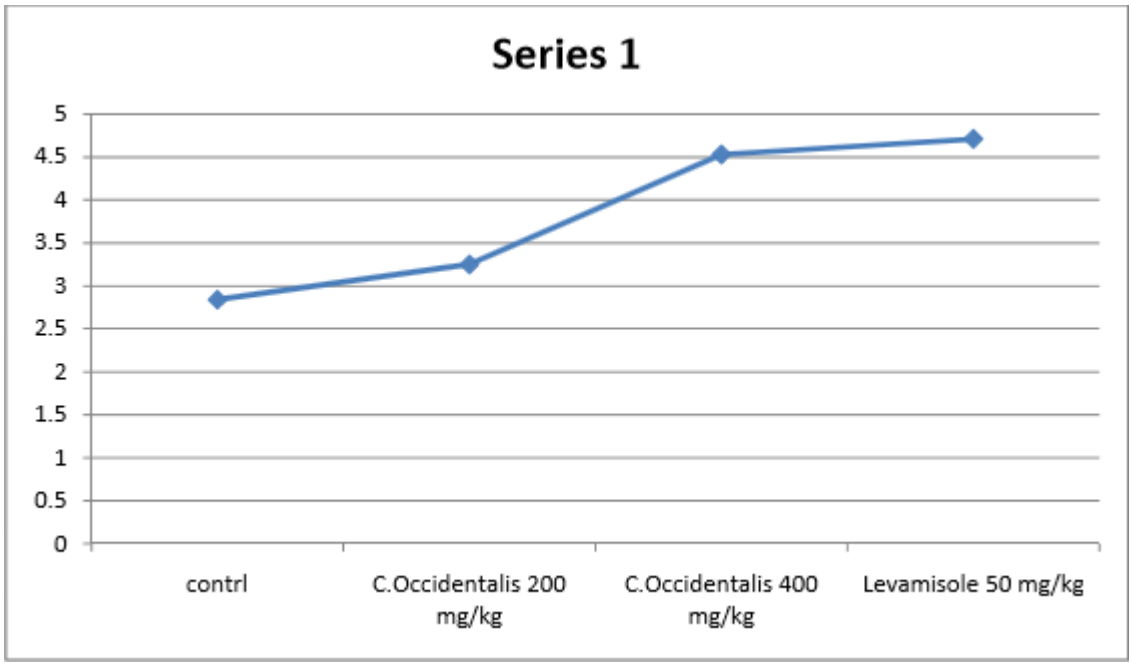
Fig. No.7.1C ***Cassia occidentalis*** **200 mg/kg**

Fig. No.7.1D ***Cassia occidentalis*** **400 mg/kg**

Fig. No.7.1E **Levamisole 50 mg/kg**

Delayed Type Hypersensitivity Reaction

Fig No. 7.2 B: Delayed Type Hypersensitivity Reaction



Cassia occidentalis reduce the paw edema by using the extract in different concentration, by the Delyed hypersensitivity reaction. In this method SRB'S antigen study shows DTH response will be increased. Mean percentage increased paw volume directly proportional to increase the concentration, nearly to the reference compound.

In order to understand the possible mechanism of action at cellular level, the effects *of Cassia Occidentalis* were studied on cell mediated host defense system by Delayed Type Hypersensitivity reactions (DTH) in rat model, since it is predominantly T-cell mediated immune reactions. DTH response is triggered by IFN-γ produced by CD4+, a Th1 (thymus derived helper cell) cells or CD8cells[72]. These cells take at least 24-72 h for the induction as well as T-cells activation which subsequently recruits monocytes and lymphocytes to the desired site for enhanced immune responses. Furthermore, DTH response is known to be initiated by interactions between antigen specific T-cells and antigen, causing the secretion of lymphokines which affects immune cells, especially macrophages. The DTH response largely represents the enhancement of lymphoproliferative events[73].In these experimental findings too, reduction of DTH response with the treatment of, Cassia Occidentalis

suggesting the increased proliferation and differentiation of Th1 cells. Such cellular events finally results in increased production of cytokines viz. IL-2, IL-6, IL-12, IFN-γ and TNF-α10-12. It is established that, IL-12 and TNF-α plays a vital role in both innate and adaptive immunity, therefore, it is worthwhile to mention here that enhanced production of TNF-α and Nitric Oxide (NO) are vital component of immune system which are participating in the protection of intracellular infections. Hence, it is likely that treatment *Cassia Occidentalis* elicit significant immunomodulatory activity[74].

7.2 Mean Humoral Antibody Titer

The effect of test extract and standard drugs on the Humoral Antibody Titer in Wister rats

Administration of aqueous extract of *cassia occidentalis* at the dose of(200& 400 mg/kg)and Levamisole 50mg/Kg treatments which were given orally for 14 days showed highly significant increase in antibody titer values compared to control group. The results are shown in below table:

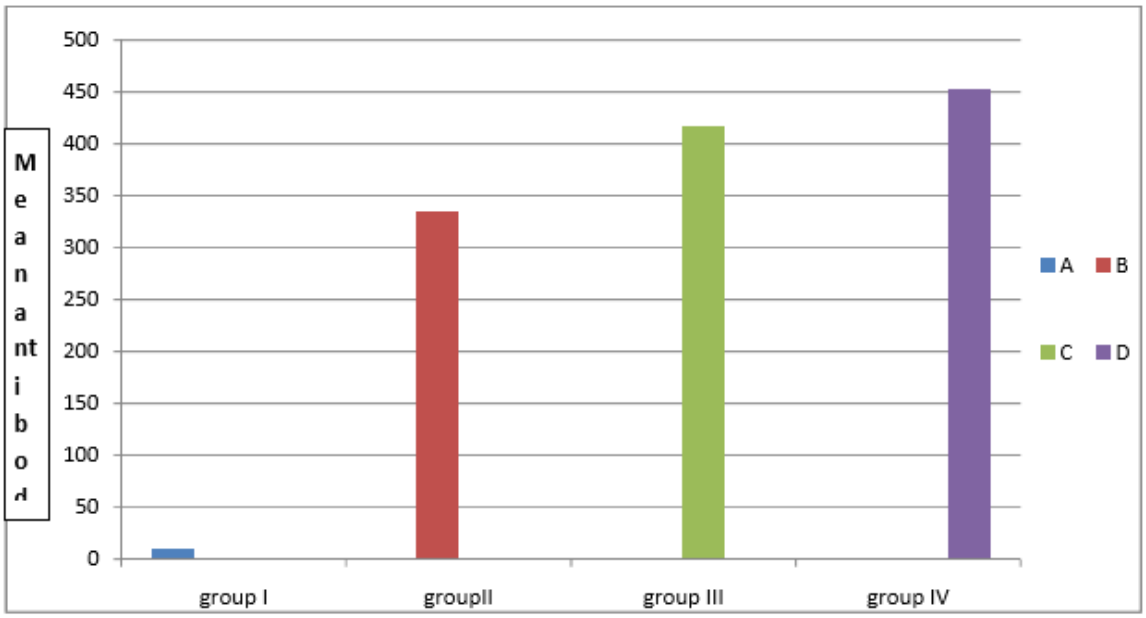
Table No 7.2: **The effect of test extract and standard drugs on the Humoral Antibody Titer in wistar rats**

Group	**Treatment**	**Dose**	**Antibody titer means ±sem**
I	Control		9±1.897
II	Test extract I	Cassia occidentalis 200 mg/kg	339.57±76.43
III	Test extract II	Cassia occidentslis 400 mg/kg	417±138.65
IV	Standard	Levamisole 50 mg/kg	452±132.14

Dunnett test and p values as significant* if $p<0.05$, highly significant** if $p<0.01$, and extremely highly significant*** if $p<0.001$ as compared to control

Humoral Antibody Titer

Fig. No 7.2A: Humoral antibody titer



Group I : Control
Group II : Cassia occidentalis 200 mg/kg
Group III : Cassia occidentalis 400 mg/kg
Group IV : Levamisole 50 mg/kg

Micro Titer Plate

Fig. No 7.2B Control

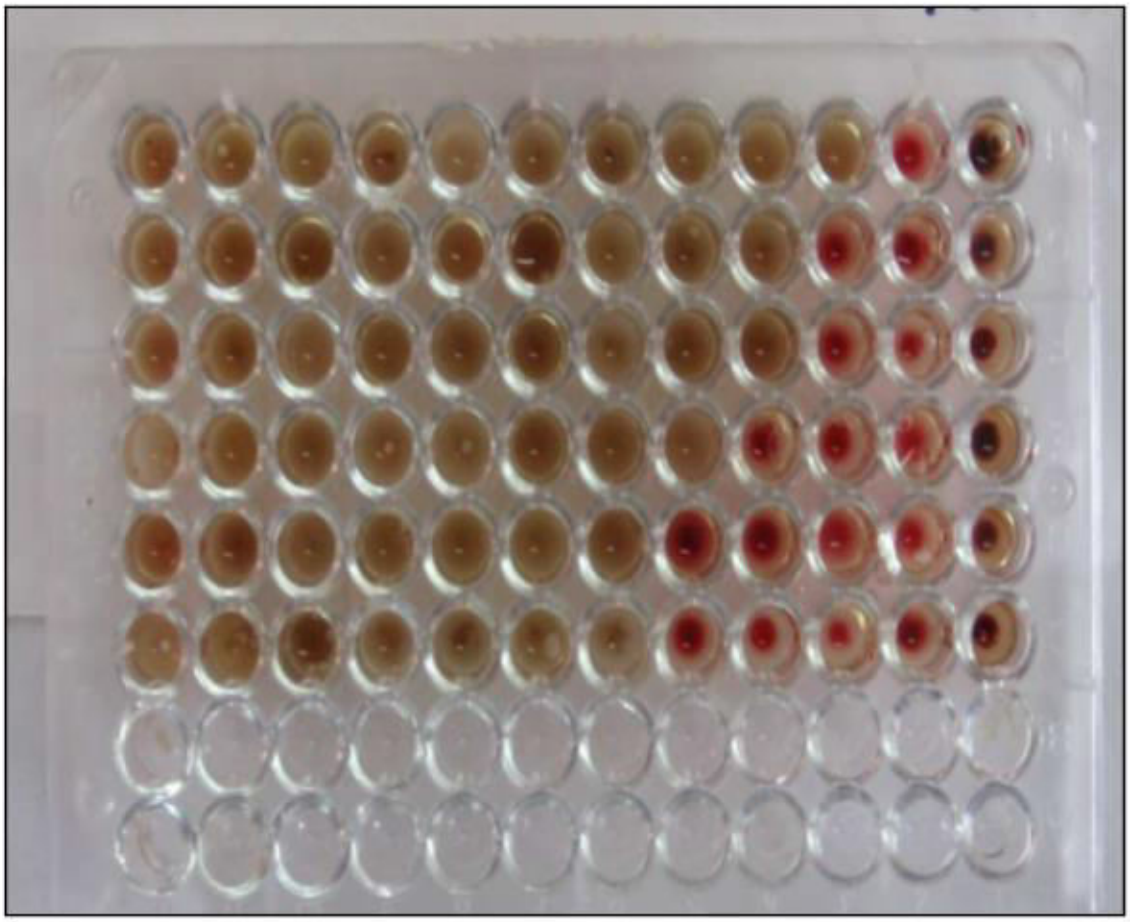


Fig. No 7.2C *Cassia occidentalis* 200 mg/kg

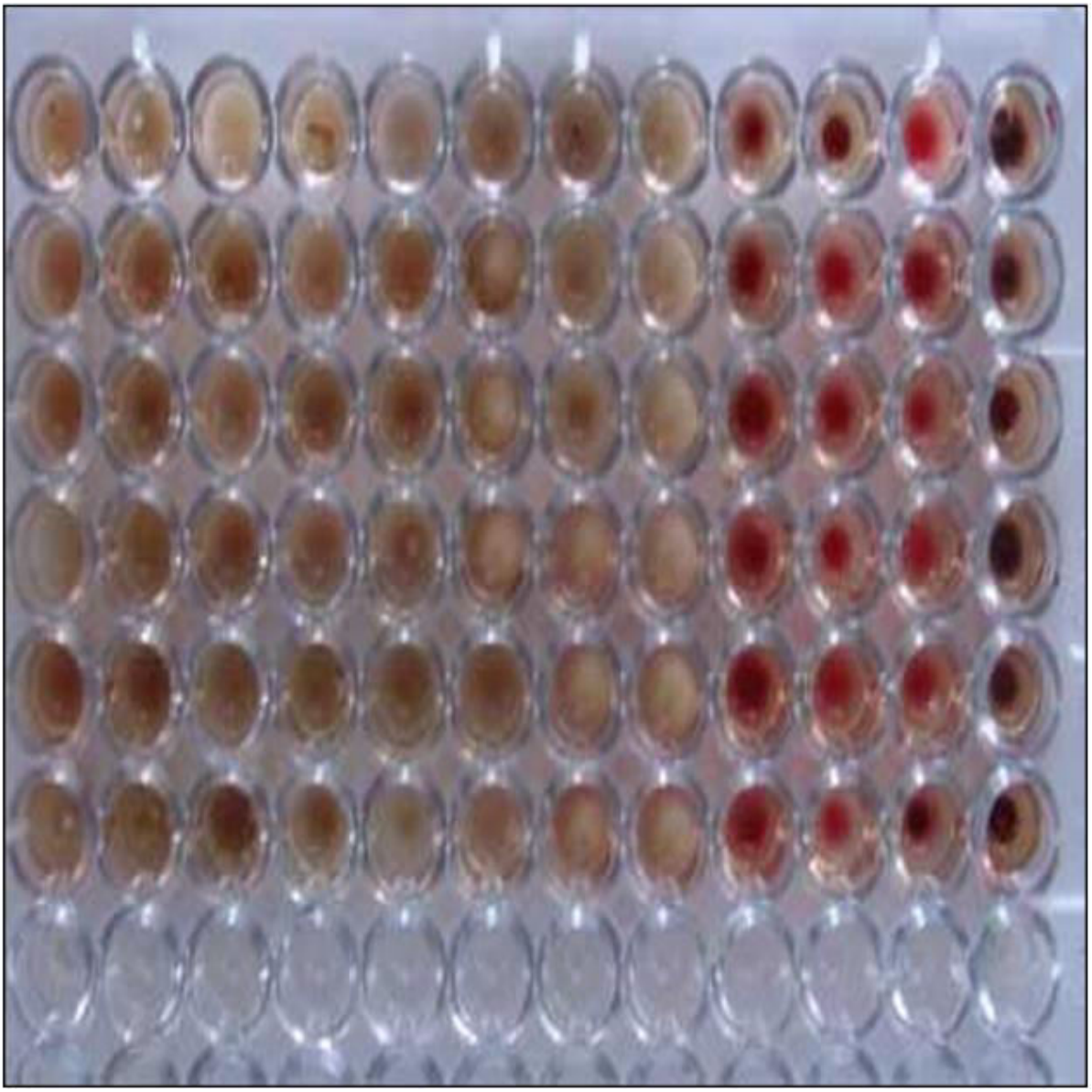


Fig. No 7.2D *Cassia occidentalis* 400 mg/kg

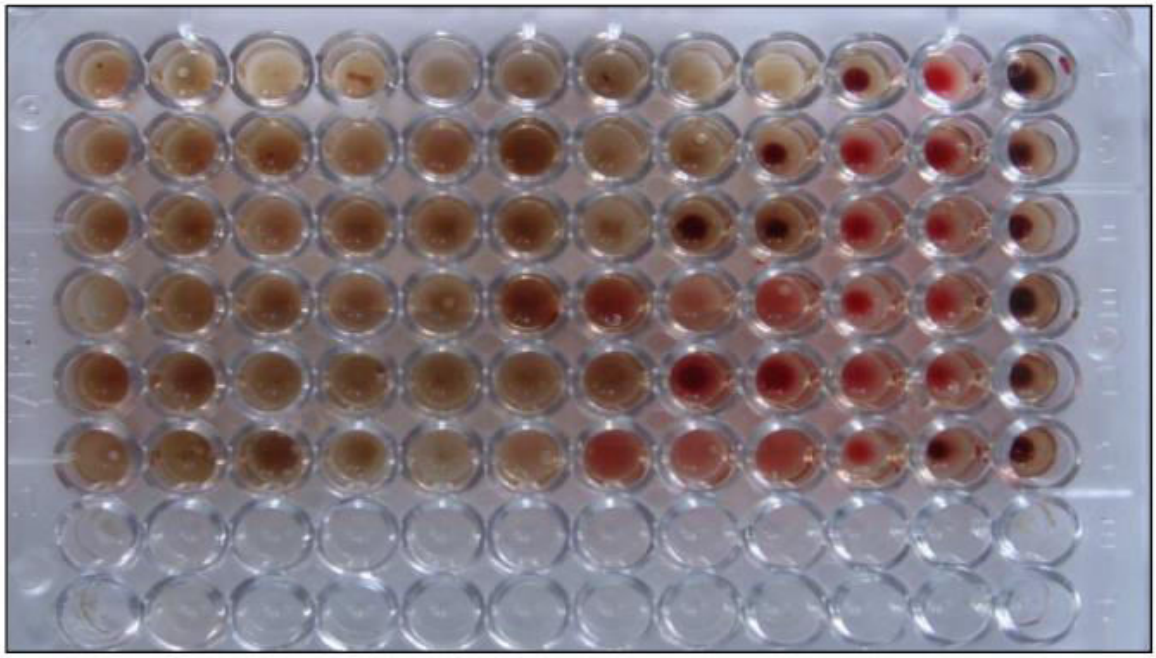
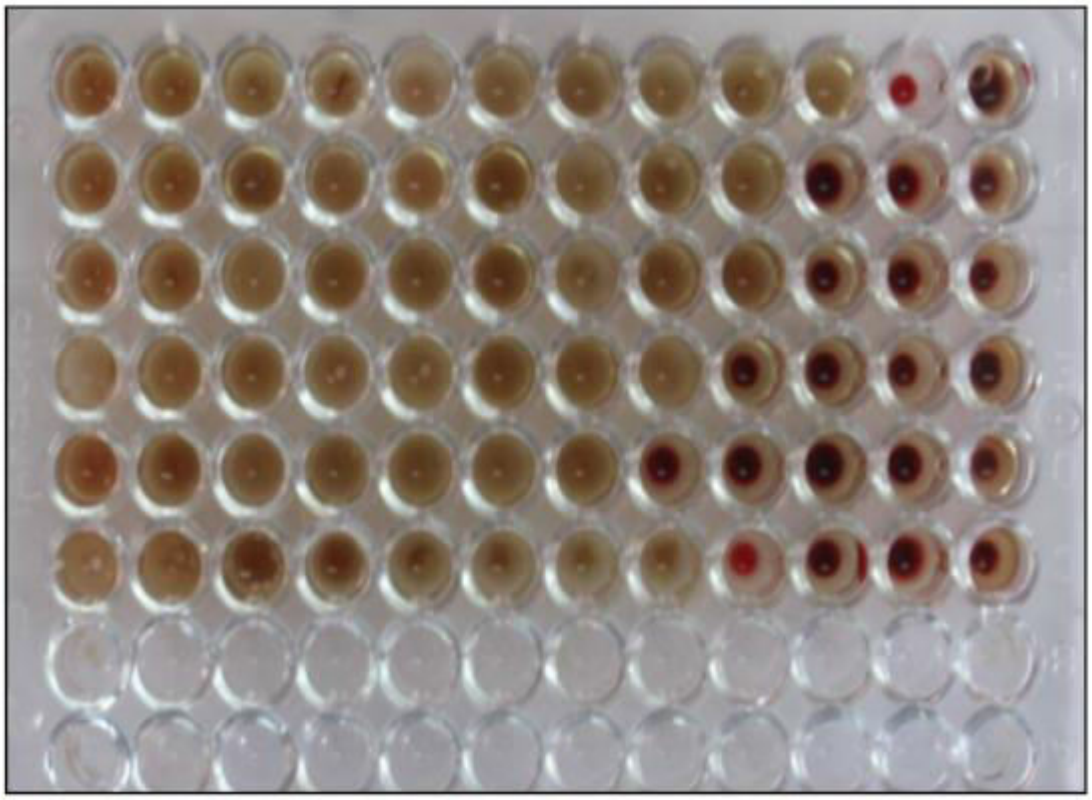
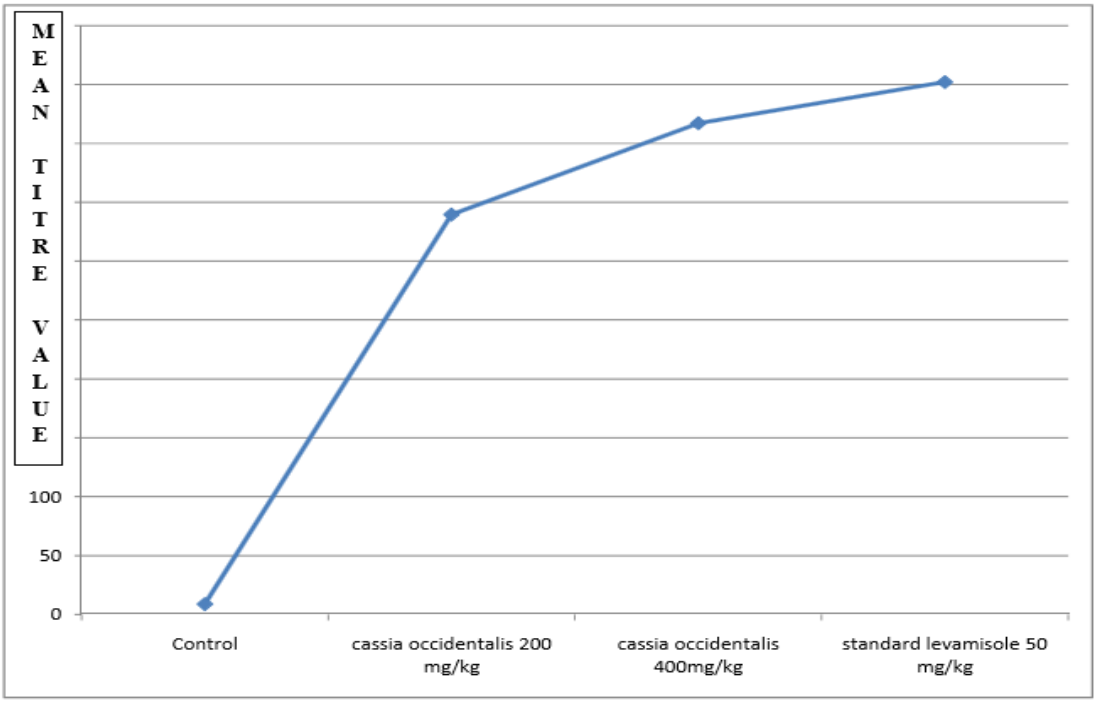


Fig. No 7.2 E Standard drug – Levamisole 50 mg/kg



Humoral Antibody Titer

Fig. No 7.3. Humoral Antibody Titer



Mean humoral antibody titer value increased proportional to the concentration. In addition, the effects of *Cassia Occidentalis* on antibody mediated humoral immune response were studied by determining antigen-antibody HA titer. Scientific evidence suggests that, there is an increased immunoglobulin formation which is considered to be an indicator of primary effector function of B-cells[75].The augmentation of humoral response to SRBCs with the treatment of *Cassia Occidentalis* was manifested in the form of increased HA titer. Such improved HA response can be explained by taking into account that IgM is more effective than IgG in agglutinating red blood cells. The increased HA titer with the treatment of *Cassia Occidentalis* likely to prove an important paradigm for evaluating improved immune reactions, that might have been achieved through the activation of lymphoid cells[76].Thus, present experimental findings with DTH and HA titer clearly demonstrated that the treatment of *Cassia Occidentalis* enhanced the proliferation of T cell and B-lymphocytes, ultimately leading to improvement of both the arms of immunity (innate and adaptive immunity).[77,78]

7.3 Total Leukocyte Count

The effect of test extract and standard drugs on Total Leukocytes in wistar rats, administration of aqueous extract of *Cassia occidentalis* at the dose of (200,400 mg/kg) and Levamisole 50mg/Kg treatments which were given orally for 14 days. The low dose of extract (200 mg/kg) did not show any effect on TLC count compared to control group, whereas the 400mg/Kg and standard drug Levamisole 50mg/Kg showed significant increase in total leukocytes count values compared to control group. The results are shown in below table:

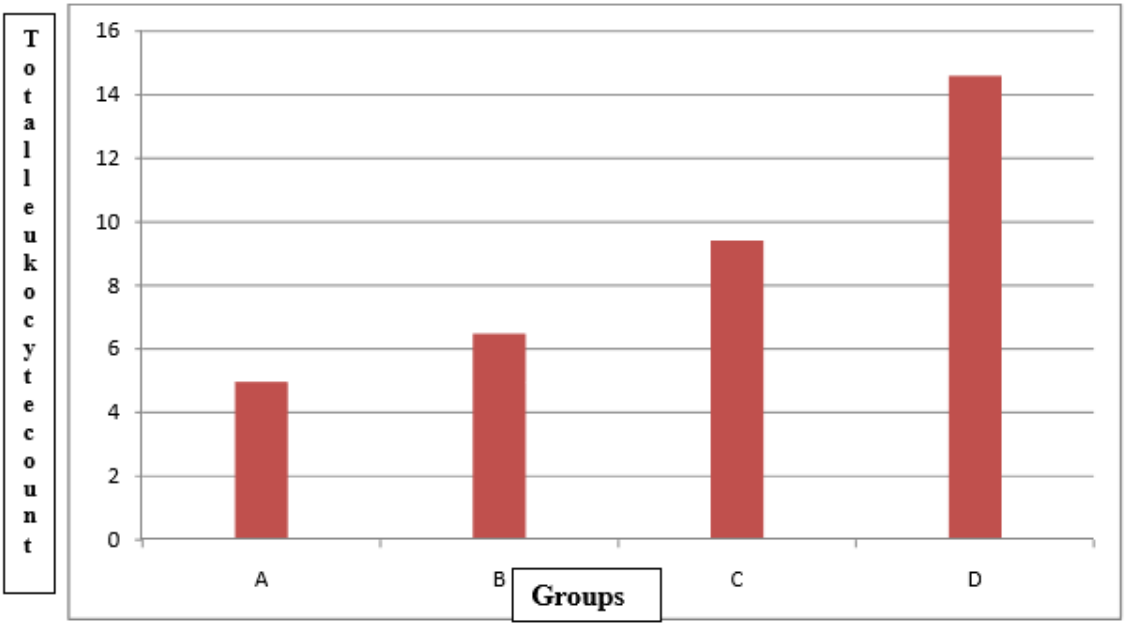
Tabel No 7.3: Total leukocyte count

Group	**Treatment**	**Dose**	**Mean leukocyte count±sem**
I	Control		5.01×10^{3}/cu.mm±0.296
II	Test extract I	Cassia occidentalis 200 mg/kg	6.89×10^{3}cu.mm±0.248*
III	Test extract II	Cassia occidentalis 400 mg/kg	9.41×10^{3}cu.mm±0.31**
IV	Standard	Levamisole 50 mg/kg	14.6×10^{3}cu.mm±0.138

Dunnett test and p values as significant* if $p<0.05$, highly significant** if $p<0.01$, and extremely highly significant*** if $p<0.001$ as compared to control.

Total Leukocyte Count

Fig No. 7.3A: Total leukocyte count



A : Control

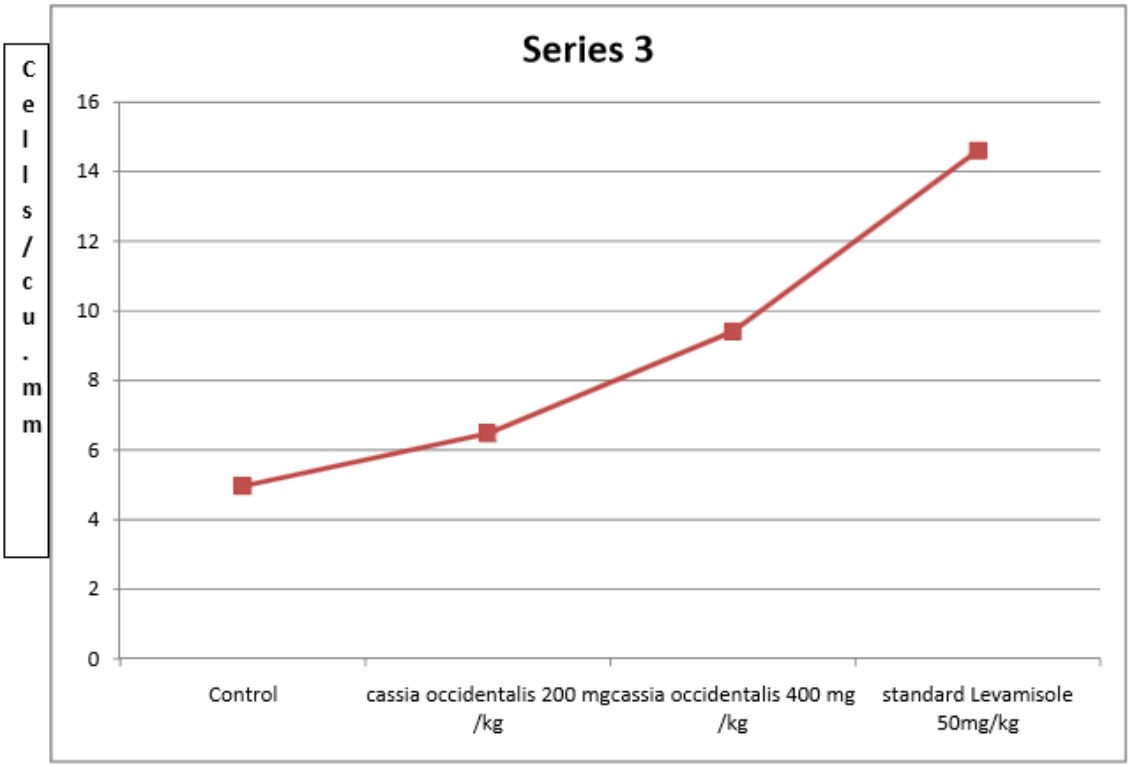
B : Cassia occidentalis 200mg/kg

C : Cassia occidentalis 400mg/kg

D : Levamisole 50 mg/*kg*

Total Leukocyte Count

Fig No. 7.4: Total leukocyte count



Value of mean humoral antibody titre, total leuckocyte count and differential leukocyte count will be increased on the basis of concentration. In present study *cassia occidentalis* increase the immunity and reduce the solid tumor volume in presence of radiation.

7.4 Differential Leukocyte Count

The effect of test extract and standard drugs on Differential Leukocytes count in Wistar rats, administration of aqueous extract of *cassia occidentalis* at the dose of (200,400 mg/kg) and Levamisole 50mg/Kg treatments which were given orally for 14 days.

a) For the differential leukocyte count, the lower dose 200mg/Kg of *cassia occidentalis* showed no increase in mean percentage of lymphocytes, Eosinophils and Neutrophils values as compared to control.

b) The results obtained from the animals that received higher dose of aqueous extract i.e. 400mg/Kg and standard drug Levamisole 50mg/Kg showed the fact there was a highly significant increase in the mean percentage of lymphocytes and significant increase in the mean percentage of Neutrophils respectively when compared to control. There is no effect in mean % increase in Eosinophils count even in 400mg/Kg dose of aqueous extract of *cassia occidentalis* and standard Levamisole-50mg/Kg compared to control group. The results were shown below table

Table no 7.4 Differential leukocyte counts

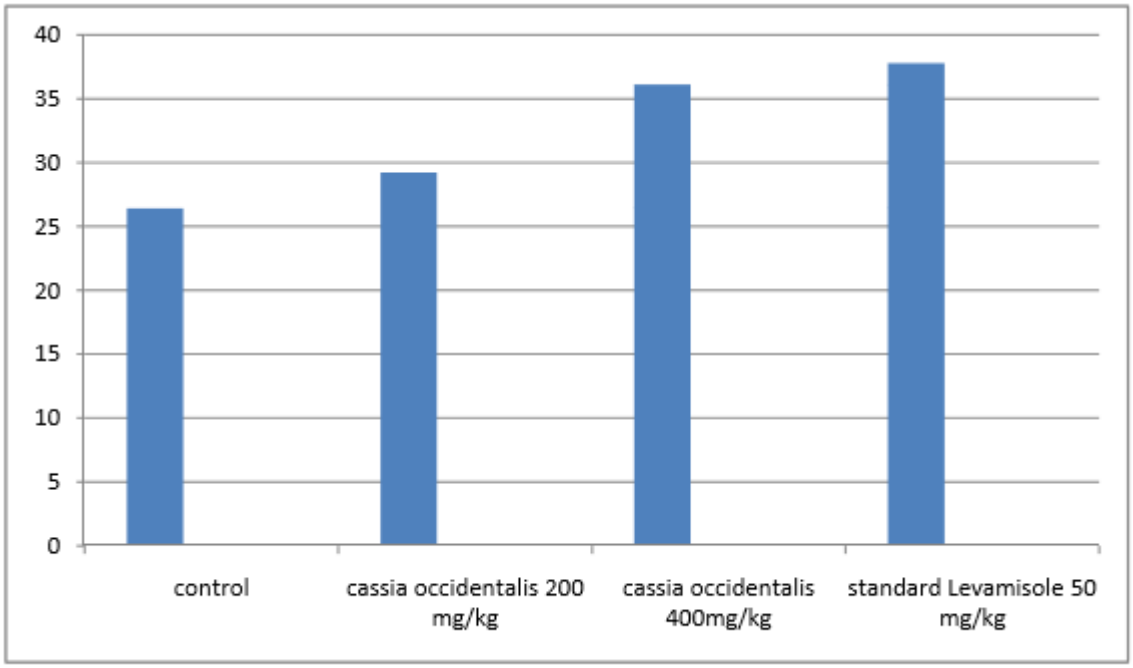
Group	**Treatment**	**Dose**	**Mean % of lymphocytes**	**Mean % of Neutrophils**	**Mean % of Eosinophils**
I	Control		26.4±0.916	66.71±0.431	5.21±0.08
II	Test extract I	Cassia occidentalis 200 mg/kg	29.21±0.431	69.54±0.42	5.60.141
III	Test extract II	Cassia occidendalis 400 mg/kg	36.1±0.34	75.08±0.546	6.5±0.01
IV	Standard	Levamisole 50 mg/kg	37.98±0.685	79.48±0.71	7.01±0.483

Dunnett test and p values as significant* if $p<0.05$, highly significant** if $p<0.01$, and extremely highly significant*** if $p<0.001$ as compared to control

Differential Leukocytes Count

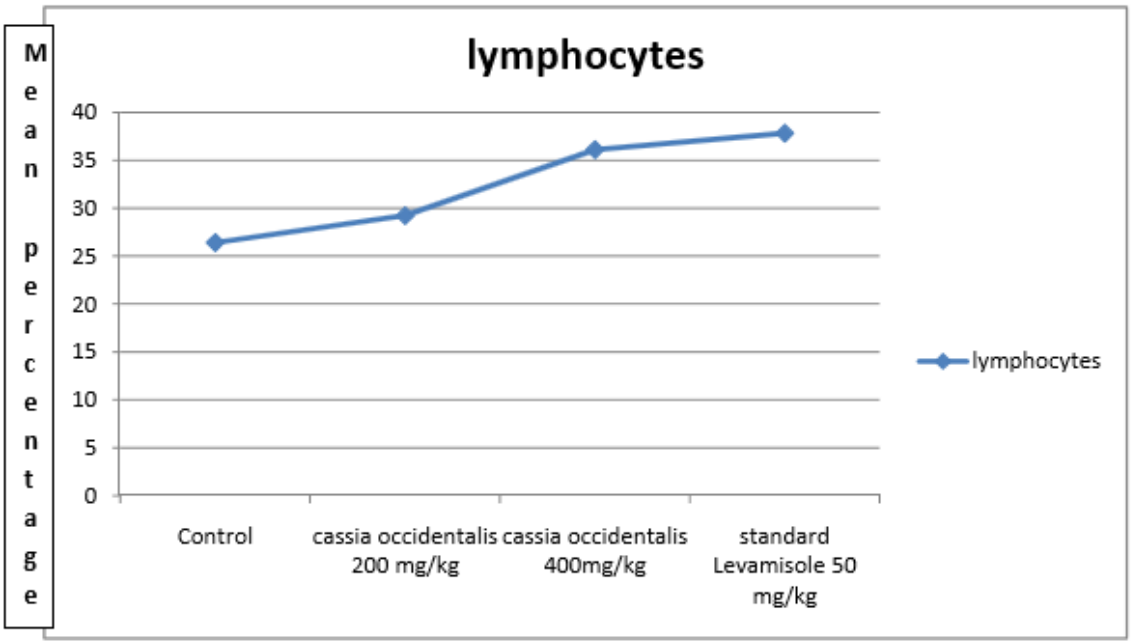
Percentage of lymphocytes

Fig. No 7.4 A Differential Leukocyte Count



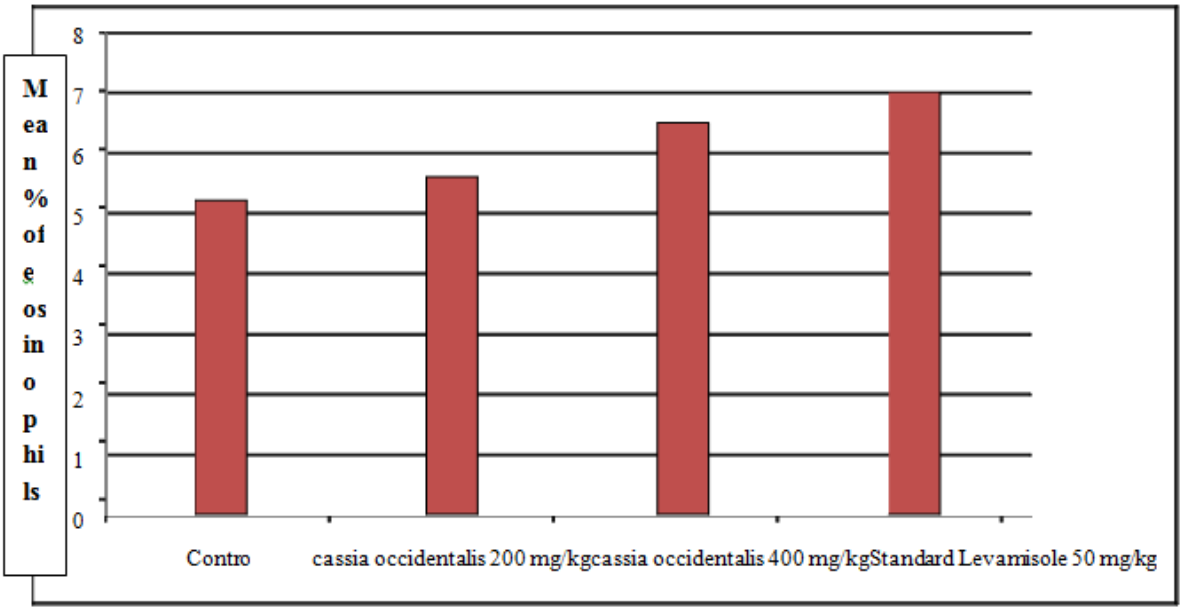
Percentage of lymphocytes

Fig. No 7.4 B Percentage of lymphocytes



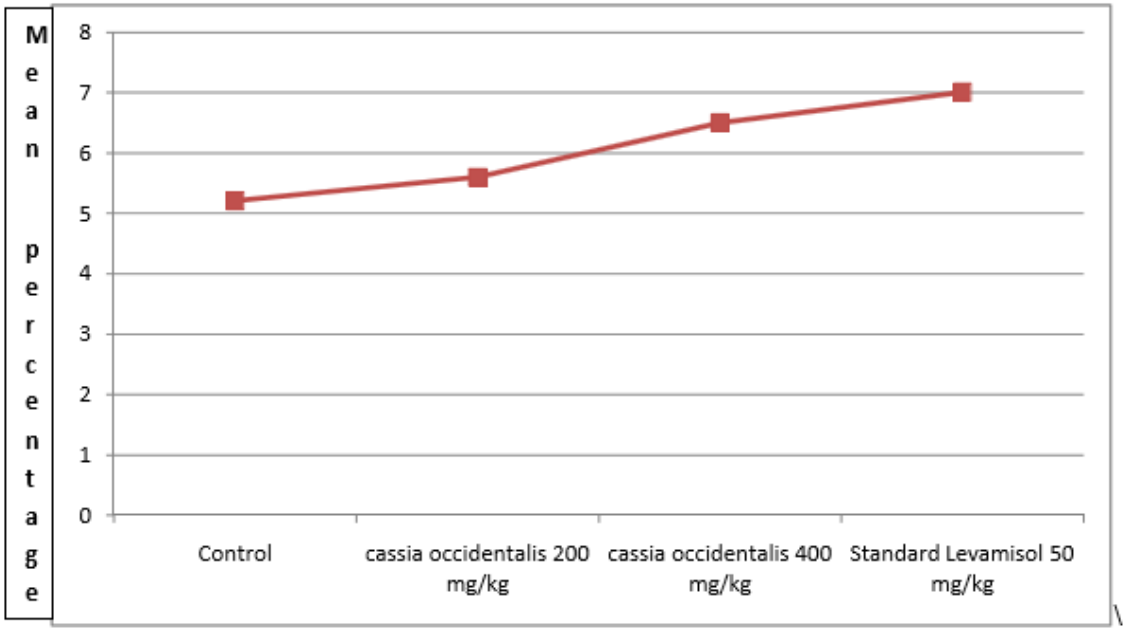
Percentage of Eosinophils

Fig.No 7.5A: Percentage of Eosinophils



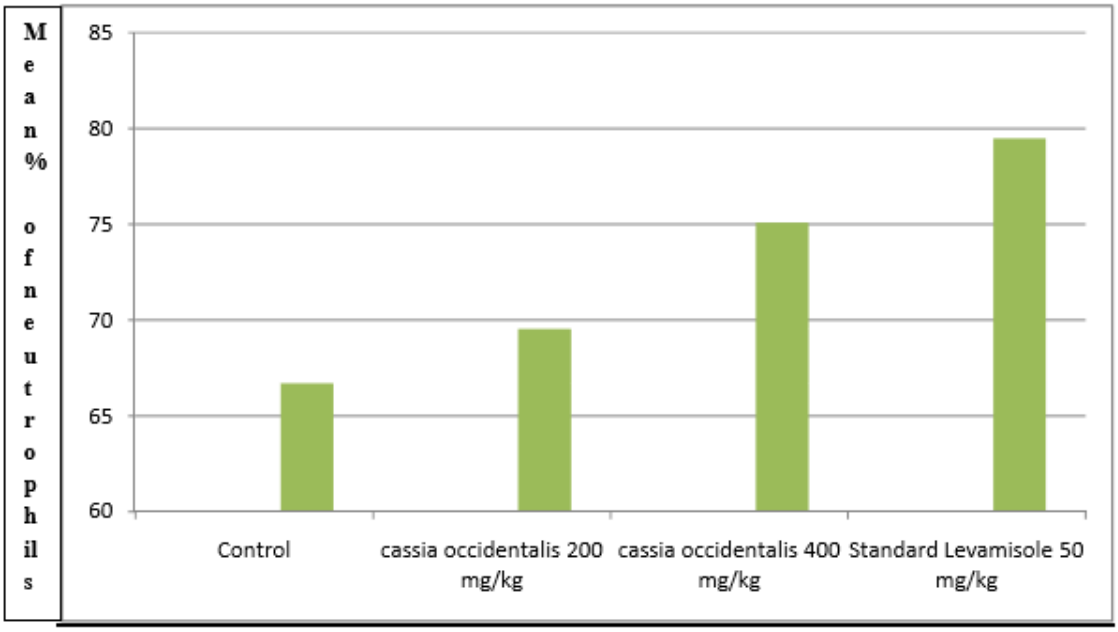
Percentage of Eosinophils

Fig.No 7.5A: Percentage of Eosinophils



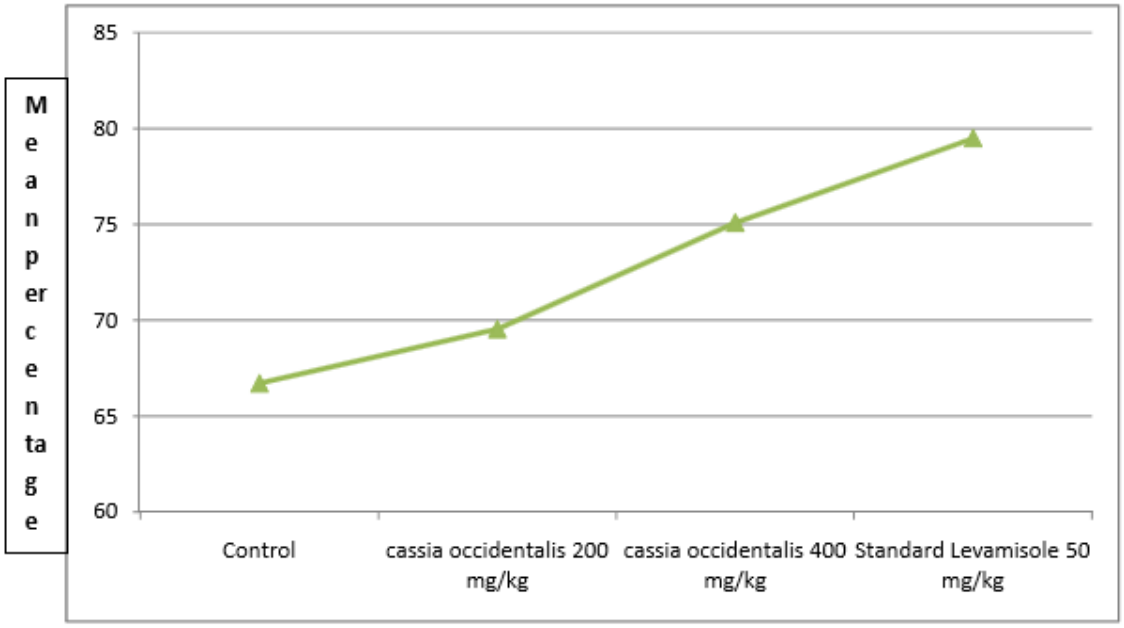
Percentage of Neutrophils

Fig. 7.6A Percentage of Neutrophils



Percentage of Neutrophils

Fig. 7.6 B: Percentage of Neutrophils



During inflammation neutrophils produce super oxide and other highly reactive oxygen products capable of inducing cell injury. Stimulation of neutrophils also induced release of granules containing lysosomal enzymes which results in cell injury. The flavanoids and steroids are present in the cassia occidentalis this may responsible to produced anticancer and immunomodulatory activity.

Modulation of the immune functions either by stimulation or suppression may help to maintain a disease-free state[65].Medicinal plants used in traditional medicines are demonstrated to modulate either cellular or humoral or both arms of the immune responses of the body[66]. Plants contain several chemical constituents, of which some constituents may have immunosuppressive activity, whereas others possess immunostimulatory action[67]. The biological activity of a plant extract is greatly dependent on its chemical nature, composition and structure of the major active metabolites[68]. Therefore, the quantitative determination of specific known bioactive compounds in herbal medicines is essential for quality control and dose determination in the toxicological and biological activity studies. The leukocytes including neutrophils, lymphocytes, monocytes, eosinophils and basophils are involved in the development of an immune response[69].Of these cells, lymphocytes are the hallmark of an immune response because of its diversity, specificity, and memory and self/non-self-recognition characteristics. All the other cells play accessory roles like activation lymphocytes, stimulation of antigen clearance by phagocytosis, secretion of various immune effector molecules. The hematopoietic action of a plant extract is demonstrated to be a direct action on the hematopoietic stem cells[70].In TLC and DLC counts, Cassia showed a dose dependent increase in TLC count and populations of monocyte and lymphocyte in rats .The possible immunostimulatory effect of Trigonella was evidenced by an increase in the TLC count with the increase in the population of monocytes and lymphocytes.[71]

Herbal drugs entitled as ‘Rasayana’ are known to possess immunomodulatory properties and known to act by stimulating both specific and non-specific immunity[79]. In DTH, antibody formation against the antigen, SRBCs requires co-operation of immune cells derived from T, B lymphocytes and macrophages[80]. Furthermore, SRBCs cause mature T-cells to differentiate into 3 distinct functioning subsets, discriminated according to the array of cytokines they produce: Th1 cells secrets IL-2, IFN-γ and tumor growth factor-β, Th2 cells produce IL-4, IL-5 and IL-6. Th0 cells

have the capacity to produce both Th1 and Th2cytokines, since they represent a stage of differentiation prior to their commitment to the Th1 or Th2 lineage.[81]

In context to the aforementioned events, macrophages are responsible for secretion of IL-1, Il-6, IFN-γ and TNF-α upon their activation15-17[82]. TNF-α is a principal mediator of acute inflammation inducing cytokines release following CYP administration. The level of TNF-α in the serum of both immune suppressed and SRBCs challenged rats were found to be reduced with the treatment which is contradictory to the above statement. Since, such findings have been reported which may be explained on the basis of biphasic action of TNF-α in immune modulation process[83]. The rats treated with *Cassia Occidentalis* also showed a decreased TNF-α level which may also be attributed to similar action

CHAPTER VIII

8. SUMMARY AND CONCLUSION

The study was undertaken to carry out the Immunomodulatory activity of aqueous extract of *Cassia occidentalis*. For the experimental work the dried leaves were powdered and extracted with distilled water and was frozen dried. The aqueous extract of Cassia occidentalis in two different doses 200mg/kg and 400mg/kg were tested for their Immunomodulatory action, out of which the higher dose of 400mg/kg showed statistically significant Immunomodulatory activity. This was evident from the different parameters that were measured.

Delayed Type Hypersensitivity Response

In this parameter the both lower dose and higher dose of the test (200 mg and 400 mg/kg) had shown significant result in increase in paw edema when compared with control. The standard drug Levamisole had shown the maximum increase in paw volume.

Humoral Antibody Titer

In this parameter both the dose of 200 mg/kg and 400 mg/kg of *cassia occidentalis* produced significant result, standard drug Levamisole at a dose of 50 mg/kg also produced significant increase in the titer value.

Total Leukocyte Count

In this parameter the lower dose of *Cassia occidentalis* 200 mg/Kg had shown no significant increase and higher dose of the aqueous extract of *Cassia occidentalis* 400 mg/Kg showed a highly significant increase in the mean total leukocyte count, as compared to control. The results were highly significant for the standard drug Levamisole 50 mg/kg as compare with the test and control group.

Differential Leukocyte Count

For the differential leukocyte count the results revealed for lower dose of *cassia occidentalis* showed no significant increase in mean percentage of lymphocytes, Eosinophils and Neutrophils increase in values as compared to control.

The results obtained from the animals that received higher dose (400mg/kg) of aqueous extract, revealed the fact there was a highly significant increase in the mean percentage of lymphocytes and significant increase in the mean percentage of neutrophils respectively when compared to control. The effect of this extract were comparable to the standard drug levamisole all the data represents the Immunostimulatory activity of aqueous extract of *Cassia occidentalis*

The results of present study revealed that the aqueous extract of leaves of *Cassia Occidentalis* generally shown immune stimulatory effect on the humoral immune function and cell mediated immunity in Wistar rats. Further, Studies are required to gain more insights into the possible mechanism of action

CHAPTER IX

9. BIBLIOGRAPHY

BIBLIOGRAPHY

1. Fariba Sharififar,Shirin Pournourmohammedali,Moslem Arabnejad. Augest 2009 Immunomodulatory activity of aqueous extract of Achillea wilhemsii c Koch in mice .Indian journel of experimental biology vol. 47, page no: 668-671.

2. U.S Patil,A.V Jaydeokar,D.D Bandawane. Immunomodulators: a pharmacological review, International Journal of Pharmacy and Pharmaceutical Sciences, vol. 4, page no.30-36.

3. Kuttan G. 2000. Immunomodulatory effect of naturally occurring sulphur-containing compounds. Journal of Ethnopharmacol., 72. page no: 93-99

4. Bodhankar S, Makare N, Rangari V. 2001 Immunomodulatory activity of alcoholic extract of Mangifera indica L. in mice. J Ethnopharmacol, 78: page no: 133-137.

5. R.Caroline Jeba, Rama Vaidyanathan, and G.Rameshkumar, 2011. Immunomodulatory activity of aqueous extract of Ocimum sanctum in rat.International journal on pharmaceutical and biomedical research.vol.2(1). page no:33-38.

6. MaheshS, Rajesh A, Amita Verma. 2013. “Immunomodulatory activity of alcoholic extract of Habenaria Intermedia in mice.” International Journal of Pharmacy and Pharmaceutical Sciences , Vol 5, P.No. 406-409

7. Tauber, A. I. A. (1994). The immune self: theory or metaphor? Immunol Today 15, 134–136

8. Baldridge, M. T. (2011), King, K. Y. & Goodell, M. A. Inflammatory signals regulate\ hematopoietic stem cells. Trends Immunol 32, 57–65

9. Wu, L. & Liu, Y.-J. (2007). Development of Dendritic-Cell Lineages. Immunity 26, 10–10

10. Anderson, G. & Takahama, Y. (2012). Thymic epithelial cells: working class heroes for T cell development and repertoire selection. Trends Immunol 33, 256–263

11. Girard, J.-P., Moussion, C. & Förster, R. HEVs, (2012). lymphatics and homeostatic immune cell trafficking in lymph nodes. Nat Rev Immunol 12, 762–773

12. Koboziev, I., Karlsson, F. & Grisham, M. B. (2010). Gut-associated lymphoid tissue, T cell trafficking, and chronic intestinal inflammation. Annals of the New York Academy of Sciences 1207 Suppl 1, E86–93

13. Mebius, R. E. & Kraal, G. (2005). Structure and function of the spleen. Nat Rev Immunol 5, 606 616

14. Takeuchi, O. & Akira, S. (2010). Pattern Recognition Receptors and Inflammation. Cell140, 805– 820

15. Hirsiger, S., Simmen, H.-P., Werner, C. M. L., Wanner, G. A. & Rittirsch, D. (2012).Danger SignalsActivating the Immune Response after Trauma. Mediators Inflamm. 2012, 1–10

16. Yao, Y., Levings, M. K. & Steiner, T. S. –(2012). ATP conditions intestinal epithelial cells to an inflammatory state that promotes components of dendritic cell maturation. Eur. J. Immunol. doi:10.1002/eji.201142213

17. Fujita, T., Matsushita, M. & Endo, Y. 202 (2004). The lectin-complement pathway–its role in innate immunity and evolution. Immunol Rev 198, 185–

18. Tarr, A. W., Urbanowicz, R. A. & Ball, J. K. (2012). The Role of Humoral Innate Immunity in Hepatitis C Virus Infection. Viruses 4, 1–27

19. Le Bourhis, L. et al(2011).. Mucosal-associated invariant T cells: unconventional development and function. Trends Immunol 32, 212–218

20. Juno, J. A., Keynan, Y. & Fowke, K. R. (2012).Invariant NKT Cells: Regulation and Function during Viral Infection. PLoS Pathog 8, e1002838

21. Sims, G. P., Rowe, D. C., Rietdijk, S. T., Herbst, R. & Coyle, A. J. HMGB1 and RAGE(2010). in Inflammation and Cancer. Annu. Rev. Immunol. 28, 367–388

22. Hardison, S. E. & Brown, G. D. (2012). C-type lectin receptors orchestrate antifungal immunity. Nat Immunol 13, 817–822

23. Weng, L. et al. (2003). Isolation and characterization of chondrolectin (ChodI), a novel C-type lectin predominantly expressed in muscle cells. Gene 308, 21–29

24. Lech, M., Avila-Ferrufino, A., Skuginna, V., Susanti, H. E. & Anders, H. J. (2010).Quantitative expression of RIG-like helicase, NOD-like receptor and inflammasome-related mRNAs in humans and mice. Int Immunol 22, 717–728

25. Rubino, S. J., Selvanantham, T., Girardin, S. E. & Philpott, D. J. (2012). Nod-like receptors in the control of intestinal inflammation. Current Opinion in Immunology 24, 398–404

26. Ireton, R. C. & Gale, M., Jr(2011). RIG-I Like Receptors in Antiviral Immunity and Therapeutic Applications. Viruses 3, 906–919

27. Artis, D. (2008). Epithelial-cell recognition of commensal bacteria and maintenance of immune homeostasis in the gut. Nat Rev Immunol 8, 411–420

28. Drouet, M., Dubuquoy, L., Desreumaux, P. & Bertin, B. (2012). Visceral fat and gut inflammation. Nutrition 28, 5–5

29. Murray, P. J. & Wynn, T. A. (2011).Protective and pathogenic functions of macrophage subsets. Nat Rev Immunol 11, 723–737

30. Nathan, C. (2006). Neutrophils and immunity: challenges and opportunities. Nat Rev Immunol 6,173–182

31. Coombes, J. L. & Powrie, F. (2008). Dendritic cells in intestinal immune regulation. Nat Rev Immunol 8, 435–446

32. Sun, J. C. & Lanier, L. L. (2011). NK cell development, homeostasis and function: parallels with CD8+ T cells. Nat Rev Immunol 11, 645–657

33. Abraham, S. N. & John, A. L. S.(2010). Mast cell-orchestrated immunity to pathogens.Nat Rev Immunol 10, 440–452

34. Godfrey, D. I., Stankovic, S. & Baxter, (2010). A. G. Raising the NKT cell family. Nat Immunol 11, 197–206

35. Kronenberg, M. & Havran, W. L. (2007). Frontline T cells: γδ T cells and intraepithelial lymphocytes. Immunol Rev 215, 5–7

36. Baumann, H. & Gauldie, J. (1994).The acute phase response. Immunol. Today 15, 74–80

37. Brogden, K. A. (2005). Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? Nature Publishing Group 3, 238–250

38. Boehm, T. (2011). Design principles of adaptive immune systems. Nat Rev Immunol 11, 307–317

39. Behrens, G. (2004). et al. Helper T cells, dendritic cells and CTL Immunity. Immunol Cell Biol 82, 84–90

40. Cerutti, A., Puga, I. & Cols, M. (2012). New helping friends for B cells. Eur. J. Immunol. 42, 1956– 1968

41. Wilson, C. B., Rowell, E. & Sekimata, M. (2009). Epigenetic control of T-helper-cell differentiation. Nat Rev Immunol 9, 91–105

42. Fathman, C. G. & Lineberry, N. B. (2007). Molecular mechanisms of CD4+ T-cell anergy.Nat Rev Immunol 7, 599–609 77

43. Patil US. Jaydeokar V. Bandawane DD. 2012 Immunomodulators: A Pharmacological Review. Int J Pharm Pharm Sci, , 4(1).page no: 30-36.

44. S. Sastry, V. Girija Sastry, B. Appalanaidu, K. Srinivas and A. 2011 Annapurna, Chemical and pharmacological evaluation of aqueous extract of seeds of Cassia occidentalis, J. Chem. Pharm. Res., , 3(2) page no: 566-575

45. Vijayalakshmi s, Ranjitha j, Devi rajeswari, Bhagiyalakshmi m 2013, pharmacological profile of cassia occidentalis, International journel of pharmacy and pharmaceutical science, vol.5, issue 3, , page no.29-33.

46. Atal, C.K., Ssharma, M.L., Kaul, A., Khajuria, A., 1986 “Immunomodulatory agents of plant origin I: preliminary screening”, Journal of Ethno Pharmacology, Vol. 18 p.no. 133-141.

47. Mohammed Musa Suleiman, 2014 Evaluation of anthelmintic activity of Nigerian ethnoveterinary plants Cassia occidentalis and Guiera senegalensis, vol. 7, july, page no. 536-541.

48. Olufunke Bolatito,shittu,Olabode,Olanrewaju olugbenga,Omemu.Phytochemical and antimicrobial screening of Spondias mombin, Senna occidentalis and Musa sapientum against Vibrio cholerae O1,Int.J.Curr.Microbiol.App.Sci (2014) 3(5).page no: 948-961Komal Arora,Allelopathic influence of cassia occidentalis l. on growth of zea mays, Ind. J. Sci. Res. And Tech. 2013 1(1).page no:15-17.

49. Komal Arora2013,Allelopathic influence of cassia occidentalis l. on growth of zea mays, Ind. J. Sci. Res. and Tech. 1(1).page no:15-17

50. Pathak,Sen., Multani,Asha S, Narayan,Satya, Kumar Virendra, Newman, Robert, 2000, “AnvirzelTM, an extract of Nerium oleander, induces cell death in human but not murine cancer cells” Anti- Cancer Drugs 11(6). page no: 455-463

51. S. Sadiq,M Shuaibu,A B Bello,S G Tureta, S Nasiru,M B Kamaru. january 2012 Phytochemistry and Antimicrobial Activities of Cassia Occidentalis Used for Herbal Remedies, journal of chemical engineering, vol. 1, no. 1, , page no.39-41.

52. M.Mohammed.M.A Aboki,H.M Saidu,O.Victor,A.Tawakalitu,S.A Maikano. April 2012, Phytochemical and Some Antmicrobial Activity of Cassia Occidentalis L, International Journal of Science and Technology, Volume 2 No.4, page no. 200-209.

53. Saba shafeen, srinath Reddy. 2012, Evaluation of antianxiety and antidepressant activity of cassia occidentalis leaves, Asian Journal of Pharmaceutical and Clinical Research Vol 5, Suppl 3, page no. 47-50.

54. A.Ravi kumar,Abbulua 2011,Antioxidant activity of ethanolic extract of cassia occidentalis against carbon tetrachloride-induced oxidative stress in wistar rats, Int. J. Chem. Sci.: 9(1),. Page no: 378-386

55. Abirami Dhandapani, Murugan Kadarkarai. 2011 HPTLC Quantification of Flavonoids, Larvicidal And Smoke Repellent Activities Of Cassia Occidentalis L. (Caesalpiniaceae) against Malarial Vectore Anopheles Stephensi Lis (Diptera: Culicidae). J Phytol phytopharmacol; 3(2). Page no: 60-72.

56. . Sini KR, Sinha BN, Karpakavalli M, Sangeetha PT. 2011 Analgesic and antipyretic activity of Cassia occidentalis Linn. Annals Biol Res; 2(1).1 page no:95-200

57. Prabh Simran Singh1*, Chetan Salwan and A.S. Mann, 2011, Evaluation of anti-diabetic activity of leaves of cassia occidentalis, International journal of research in pharmacy and chemistry, 1(4), page no 903-913.S.

58. Emmanuel.M Sheeba Rani,M Rraja Sreekanth Oct-Dec.2010,.Antidiabetic activity of cassia occidentalis linn. Instreptozotocin-induced diabetic rats: a dose dependent study,International Journal of Pharma and Bio Sciences,Vol.1/Issue-4/ page no .B-14 –B-25.

59. Bilal Bin-Hafeez, Iqbal Ahmad, Rizwanul Haque, Raisuddin S. 2001 Protective effect of Cassia occidentalis L. on cyclophosphamideinduced suppression of humoral immunity in mice. J Ethnopharmacol; 75(1).1 page no:3-18

60. Kokate CK. 2008 Text book of Pharmacognosy. 43rd edition. New Delhi; VallabhaPrakashan.page no:6.13-6.17

61. OECD (2001). Test Guideline 423. Acute Oral Toxicity – Acute Toxic Class method.

62. Shukla S, Mehta A, John J, Mehta P, Vyas SP. 2009 Immunomodulatory activities of the ethanolic extract of Caesalpinia bonducella seeds. J Ethnopharmacol; 125:252-6.

63. Plaeger SF. 2003 Clinical immunology and traditional herbal medicines. Clin Diagn Lab Immunol; 10:337-8.

64. Tiwari U, Rastogi B, Singh P, Saraf DK, Vyas SP. 2004 Immunomodulatory effects of aqueous extract of Tridax procumbens in experimental animals. J Ethnopharmacol; 92:113-9. 79

65. Ghule BV, Yeole PG. 2012; In vitro and in vivo immunomodulatory activities of iridoids fraction fromBarleria prionitis Linn. J Ethnopharmacol 141:424-31.

66. Biedermann TR, Mailhammer A, Mai C, Sander A, Ogilivie F, Brombacher K, et al. 2001 Reversal of established delayed-type hypersensitivity reactions following therapy with IL-4 or antigen specific Th2 cells. Eur J Immunol; 31:1582-91.

67. Biedermann, T.R., A. Mailhammer, C. Mai, A. Sander and F. Ogilivie et al., 2001. Reversal of established delayed-type hypersensitivity reactions following therapy with IL-4 or antigen specific Th2 cells. Eur. J. Immunol., 5: 1582-1591

68. Osadebe, P.O. and E.O. Omeje, 2009. Comparative acute toxicities and immunomodulatory potentials of five Eastern Nigeria mistletoes. J. Ethnopharmacol., 126: 287-293

69. .Finkelman, F.D., J. Holmes, I.M. Katona, J.F. Urban and M.P. Beckmann et al., 1990.Lymphokine control of In vivoimmunoglobulin isotype selection. Annu. Rev. Immunol., 8: 303- 333

70. .Malik, F., J. Singh, A. Khajuria, K.A. Suri and N.K. Satti et al., 2007. A standardized root extract of Withania somnifera and its major constituent withanolide-A elicit humoral and cellmediated immune responses by up regulation of Th1-dominant polarization in BALB/c mice. Life Sci., 80: 1525-1538

71. .Andhare, R.N., M.K. Raut and S.R. Naik, 2012. Evaluation of antiallergic and anti-anaphylactic activity of Ethanolic Extract ofSanseveiria trifasciata leaves (EEST) in rodents. J. Ethnopharmacol., 142: 627-633

72.Naik, S.R. and A. Hule, 2009. Evaluation of immunomodulatory activity of an extract of andrographolides from Andographis paniculata. Planta Medica, 75: 785-791

73..Mitsuoka, A., B. Mitsuo and S. Morikawa, 1976. Enhancement of delayed hypersensitivity by depletion of suppressor T cells with cyclophosphamide in mice. Nature, 262: 77-78

74 .Han, S.B., Y.H. Kim, C.W. Lee, S.M. Park and H.Y. Lee et al., 1998. Characteristic immunostimulation by angelan isolated from Angelica gigas Nakai. Immunopharmacology, 40: 39-48 80

75..Arend, W.P. and J.M. Dayer, 1990. Cytokines and cytokine inhibitors or antagonists in rheumatoid arthritis. Arthritis Rheumatism, 33: 305-315

76. Gurne, P.A., B.L. Zuraw, J.H. Vaghan, D.A. Carson and M.N. Lotz, 1989. Synovium as a source of interleukin 6 in vitro.Contribution to local and systemic manifestations of arthritis. J. Clin. Invest., 83: 585-592

77..Thorbecke, G.J., R. Shah, C.H. Leu, A.P. Kuruvilla, A.M. Hardison and M.A. Palladino, 1992. Involvement of endogenous tumor necrosis factor α and transforming growth factor β during induction of collagen type II arthritis in mice. Proc. Natl. Acad. Sci. USA., 89: 7375-7379

78. Spelman, K., J. Burns, D. Nichols, N. Winters, S. Ottersberg and M. Tenborg, 2006. Modulation of cytokine expression by traditional medicines: A review of herbal immunomodulators. Altern. Med. Rev., 11: 128-150

79. Veterinary clinical pathology-Gasti A. satry

80. Animal Hematotoxicology, A Practical Guide for Toxicologist and Biomedical Researches by G.O.Evans Vol.1.

81. A. Text book of animal husbandry G. C. Banerjee VIIth edition. Indian medicinal plants.Kirtikar & Basu Vol.1.

82. Textbook of Pharmacognosy by Kokate Vth edition.

83. Handbook of medical laboratory technology IInd edition Robert H. carman.

84. Textbook microbiology IVth edition by R. Ananthanarayanan & CKJ Panikar.

85. Textbook of Pharmacology by Rang & dale IIIrd edition.

86. Textbook of immunology by R.S Chituram & V.S. Goram. VIIth edition.

87. Textbook of Pharmacology by Sathoshkr IVth edition.

88. Textbook of medicinal pharmacology by Ramann.C & Sri. Surenderisingh IIIrd edition.

89. Kulkarni SR, Karandevs. 1998 Study of Immunostimulant activity Indian Drugs: 35:427 33

90. Textbook of Pharmacognosy by Trease & Evans IVth edition. 81

91. C.C Chatterjee1972, "Human physiology" Vol. 1 Editor medical allied agency m.851/1 Mahatma ganthi road, Calcutta-9. II edition p.no 174-176.

92.Indian journal of pharmacology Vol. 34(2002) p.no. 194-197.

93.Nayampalli SS, Desai NK, Ainapure SS1986. Ind. J. Pharmacol; 18:50.

94. Al-Hindawi MK, al-Khafaji S, J1992. Ethnopharmacol; 37:113-6.

95..Pallabi DE, Dasgupta SC, 1998 Gomes A Immuno Potentiating activity of immune-21; A Polyherbal product. Indian J Pharmacol; 30:163-8.

96.Moudgil KD, Singh AK, Talwar GP, 1993 A handbook of practical and clinical immunology. New Delhi; CBS Publishers,: 194.

97.Park's (text book of preventive and social medicine) 7th edition by K.Park Pg. 81-83.

98.Parrotta A John. 2001 Healing plants of peninsular India.Wallingford, U K: CABI Publishing;. p. 131-132.

99.Rastogi RP and Mehrotra BN. 2006 Compendium of Indian medicinal plants. Vol2 (1970-1979). New Delhi, India:Central Drug Research, Lucknow and NISCAIR;. p.521.

100.http://plants.usda.gov

101.Selvanayagam ZE, Gnavavendhan SG, Balakrishna K, Rao RB, Ali SU. 1995 Survey of medicinal plants with antisnakevenom activity in Chengalpattu district, Tamilnadu, India. Fitoterapia; 66 (6): 488-494.

102.Dutta A, Ghosh S. 1947 Chemical examination of Daemia extensa. I. J Amer Pharm Ass Sci Ed a;36: 250-252.

103.WHO, IUCN, WWF. Guidelines on the conservationof medicinal plants. Switzerland: IUCN Gland; 1993.

104.Kotnis MS, Patel P, Menon SN, Sane RT. Rene protective effect of Hemisdesmus indicus, a herbal drug used in Gentomicin-induced renal toxicity. Nephrology (Carlton) 2004; 3: 142-152.82

105.Bhagwati Uniyal 2003. Utilization of medicinal plants by the rural women of Kulu, Himachal Pradesh. Indian J Trad Knowledge; 2(4): 366-370.

106.Dahanukar SA, Kulkarni AR, Rege NN. 2000 Pharmacology of medicinal plants and natural products. Indian J Pharmacol; 32: S81-S118.

107.. Fathman, C. G. & Lineberry, N. B. (2007). Molecular mechanisms of CD4+ T-cell anergy.Nat Rev Immunol 7, 599–609

108. Romagnani, S. (1997).The Th1/Th2 paradigm.Immunol.Today 18, 263–266

109. Miossec, P. & Kolls, J. K. (2012). Targeting IL-17 and TH17 cells in chronic inflammation. 1–14 doi:10.1038/nrd3794

110. Bilate, A. M. & Lafaille, J. J. (2012). Induced CD4 +Foxp3 +Regulatory T Cells in Immune Tolerance. Annu. Rev. Immunol. 30, 733–758

111.. Smith, K. G. C. & Clatworthy, M. R. (2010). FcγRIIB in autoimmunity and infection: evolutionary and therapeutic implications. Nat Rev Immunol 10, 328–343

112. Stavnezer, J., Guikema, J. E. J. & Schrader, C. E. (2008). Mechanism and Regulation of Class Switch Recombination. Annu. Rev. Immunol. 26, 261–292

113. Kaech, S. M., Wherry, E. J. & Ahmed, R. (2002) VACCINES: EFFECTOR AND MEMORY T-CELL DIFFERENTIATION: IMPLICATIONS FOR VACCINE DEVELOPMENT. Nat Rev Immunol 2, 251–262

114.www.immunology.com\ 83